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Ponce et al.

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(54) **BIOLOGICAL INDICATORS, AND SYSTEMS AND METHODS FOR DETERMINING EFFICACY OF STERILIZATION**

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(73) Assignee: **STERITEC PRODUCTS MFG. CO., INC.**, Englewood, CO (US)

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C12Q 1/00 (2006.01)
C12M 3/00 (2006.01)
(Continued)

(52) **U.S. Cl.**
CPC ***C12Q 1/22*** (2013.01); ***C12M 37/06*** (2013.01)

(58) **Field of Classification Search**
CPC C12M 37/06; C12Q 1/22
See application file for complete search history.

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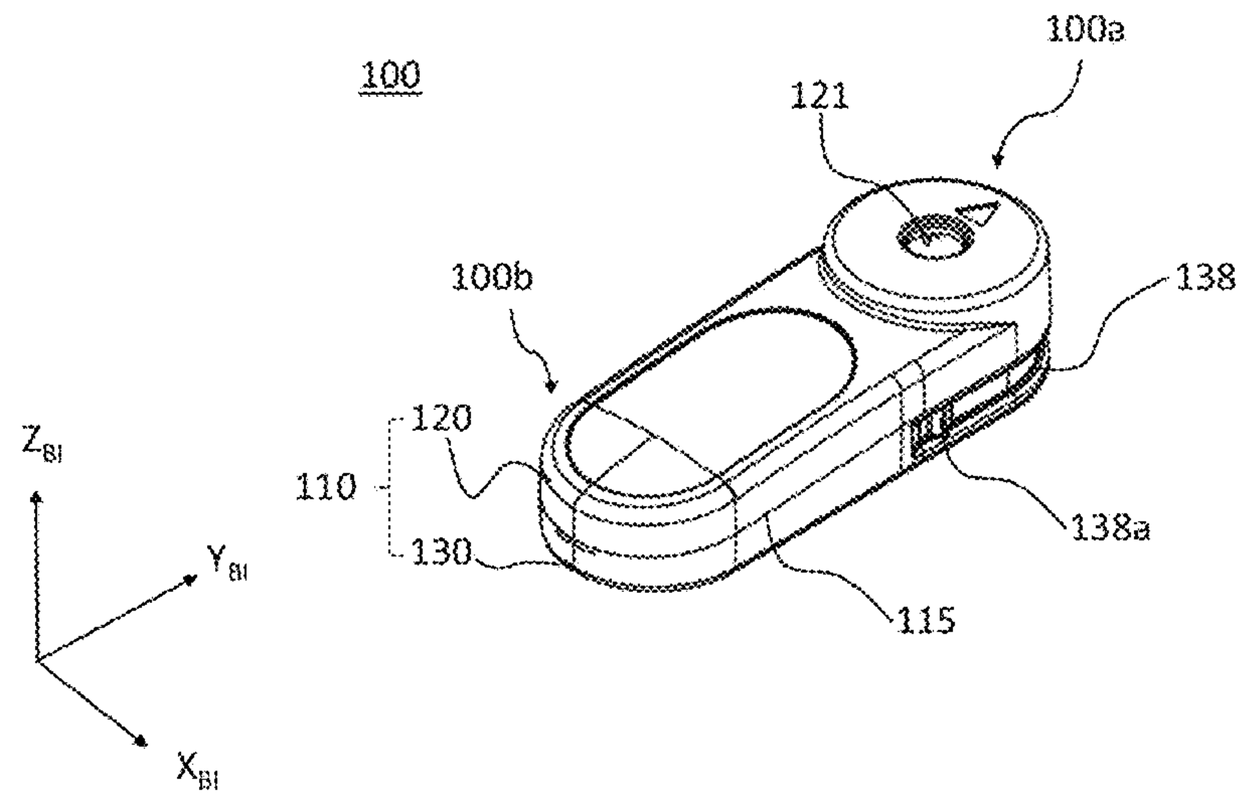
Primary Examiner — Nathan A Bowers
(74) *Attorney, Agent, or Firm* — Lewis Roca Rothgerber Christie LLP

(57) **ABSTRACT**

A biological indicator includes: a BI housing; a germinant container inside the BI housing and housing a germinant composition; a germinant releaser configured to release the germinant composition from the germinant container; a germinant releaser support supporting the germinant releaser and configured to bring the germinant releaser into contact with the germinant container upon application of a force to the germinant releaser support or the germinant container; a first spore carrier inside the BI housing, the first spore carrier having a plurality of spores deposited at a first surface thereof; and an imaging window at a first surface of the BI housing. A BI reader is configured to detect and quantify the presence of live spores in the BI, and includes an excitation source, a camera for capturing images of the spores over time, and a processor for analyzing the images to determine the presence of live spores.

21 Claims, 59 Drawing Sheets



(51) **Int. Cl.**
C12Q 1/22 (2006.01)
C12M 1/12 (2006.01)

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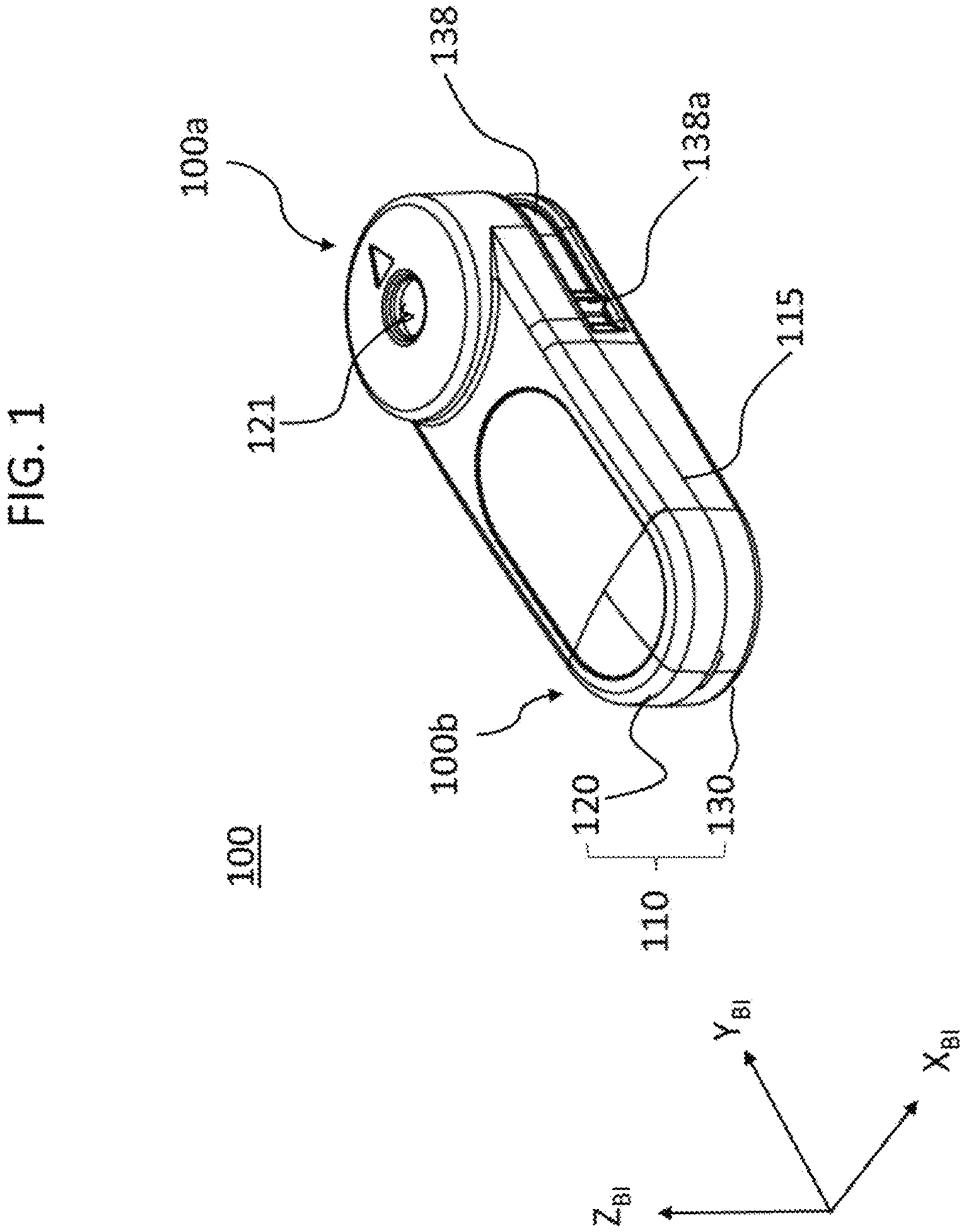
FIG. 1



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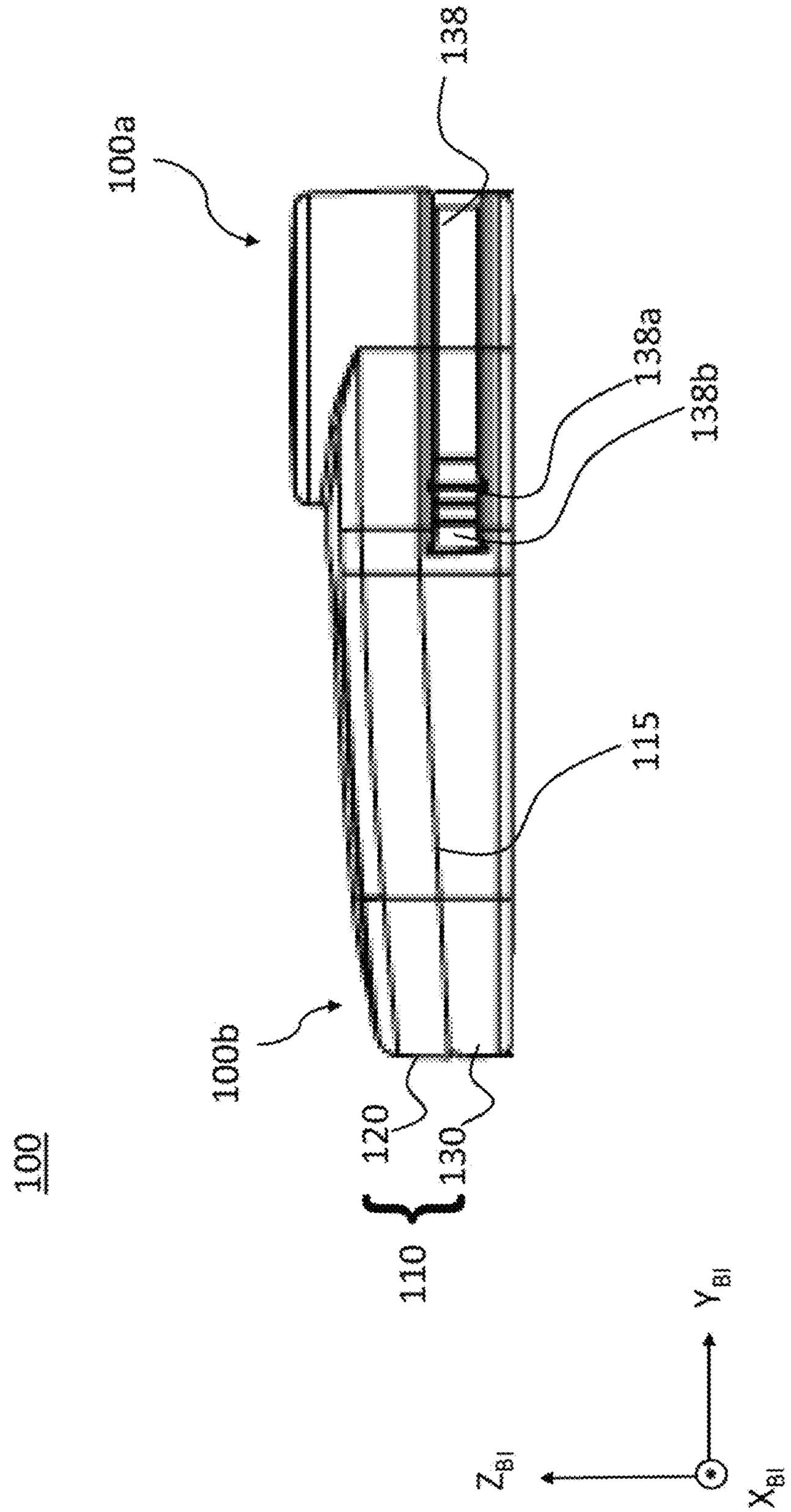


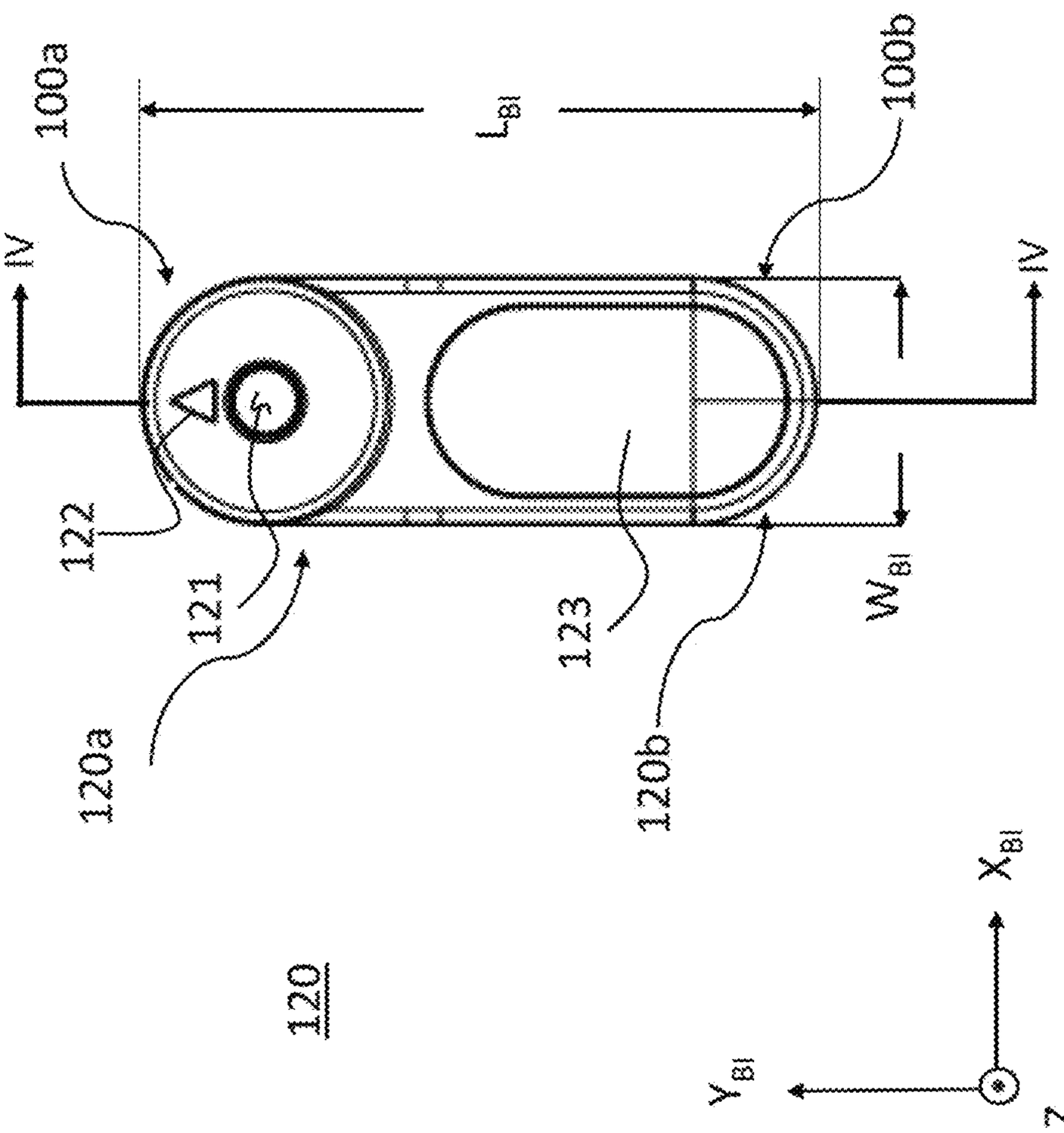
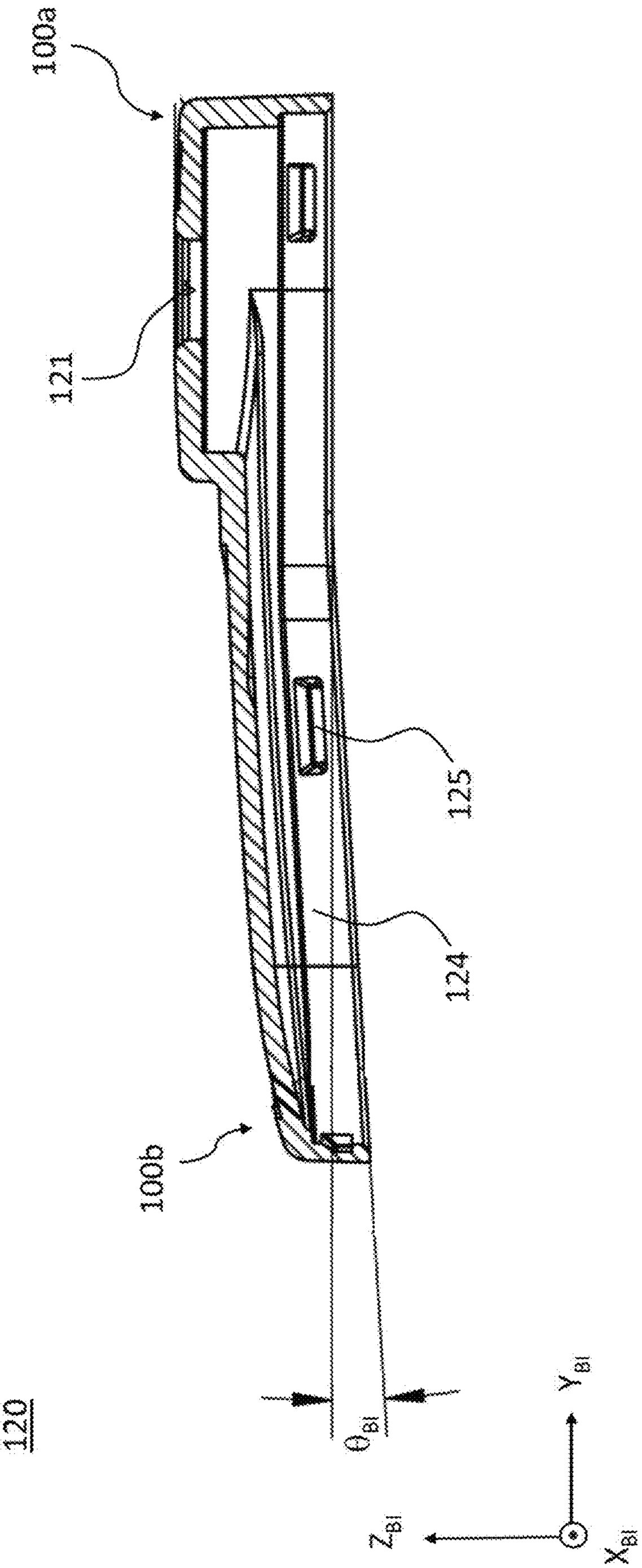


FIG. 3

FIG. 4



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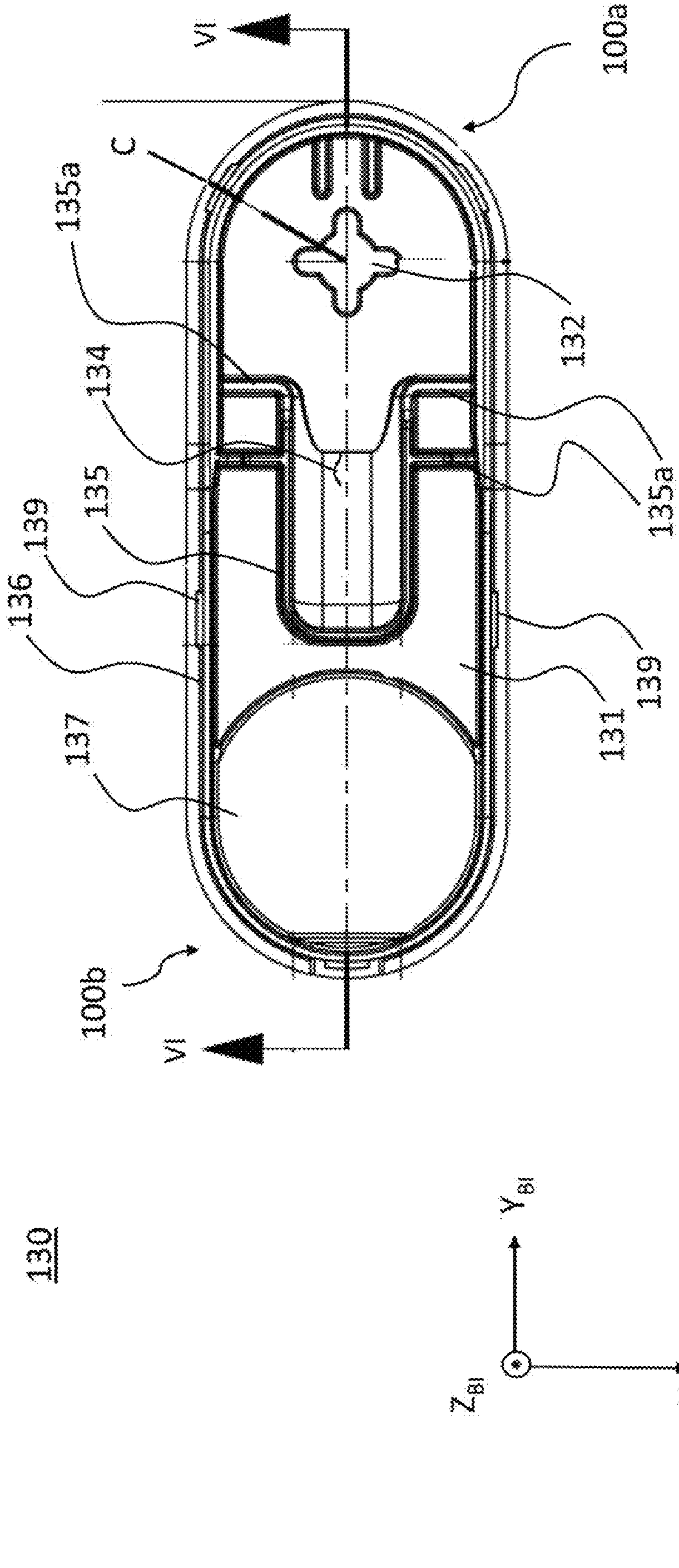

VI
100a
C
135a
134
135
139
136
137
132
135a
139
131
139
100b
VII
130
Y_{BI}
Z_{BI}
X_{BI}

FIG. 6

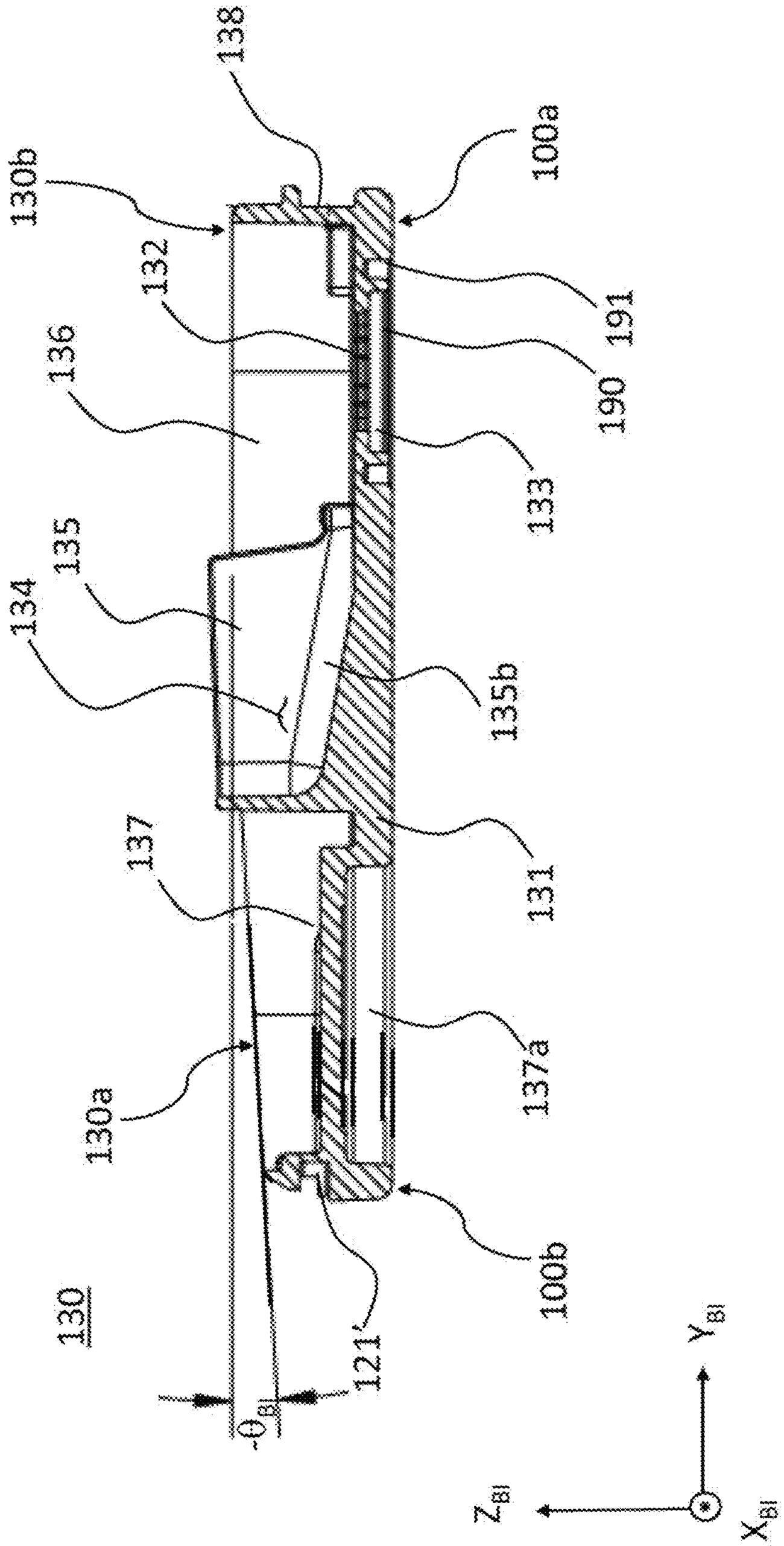

138
130b
100a
132
191
136
190
133
135
134
135b
137
131
130a
137a
130
121'
100b
$-D_{BI}$
Y_{BI}
Z_{BI}
X_{BI}

FIG. 7

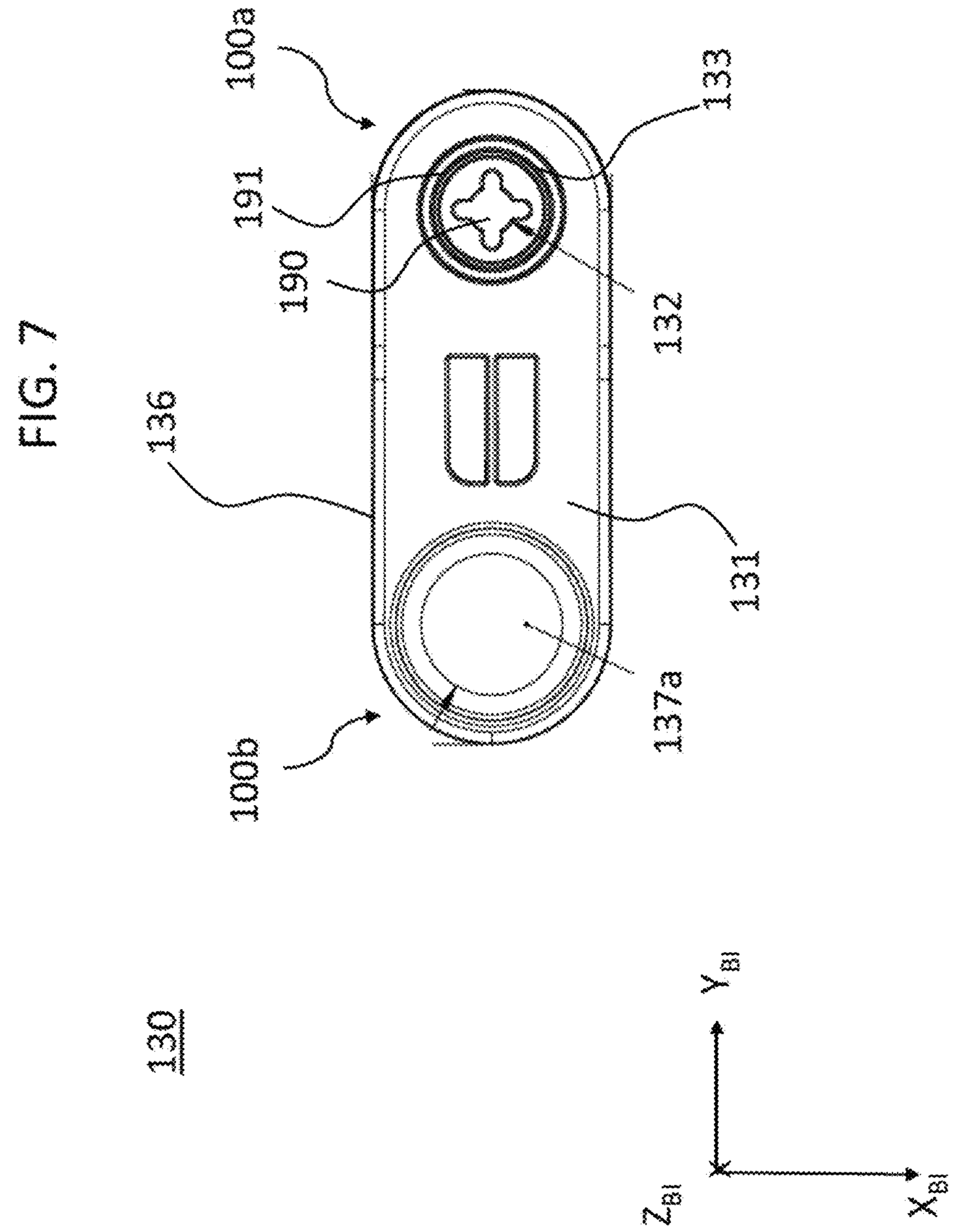


FIG. 8

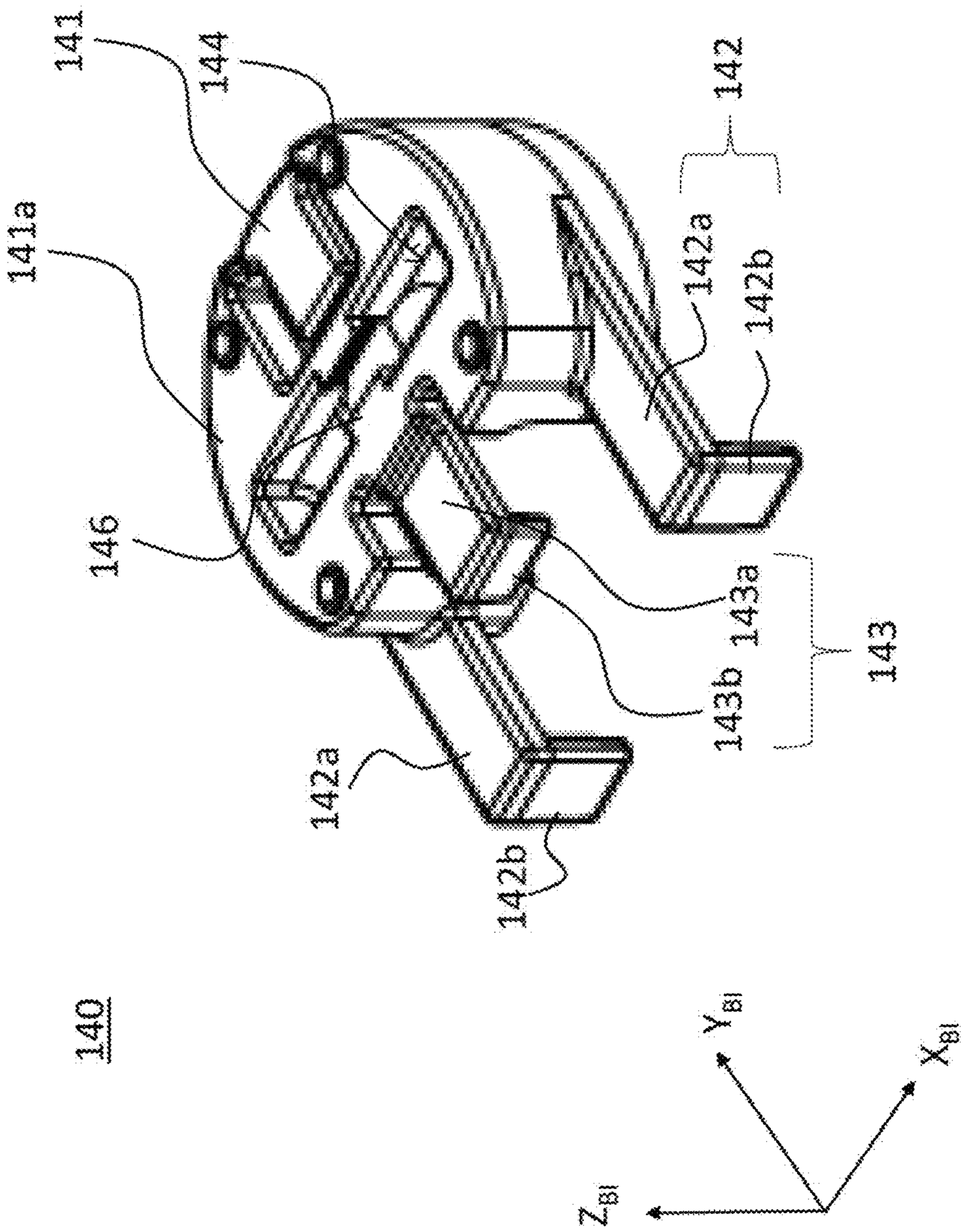
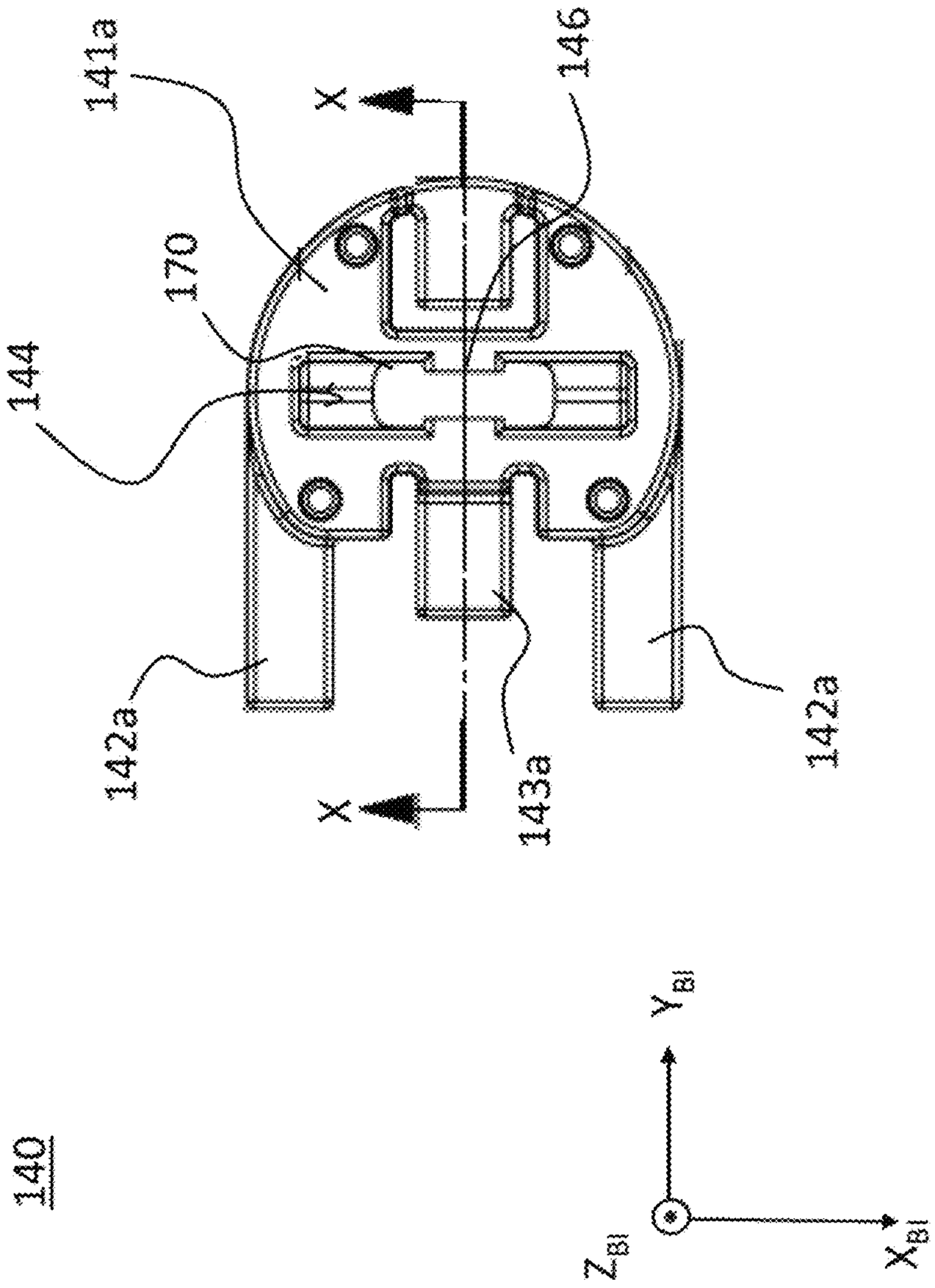

140
141
144
142
141a
142a
142b
146
143a
143b
143
142a
142b
Y_{8I}
X_{8I}
Z_{8I}

FIG. 9



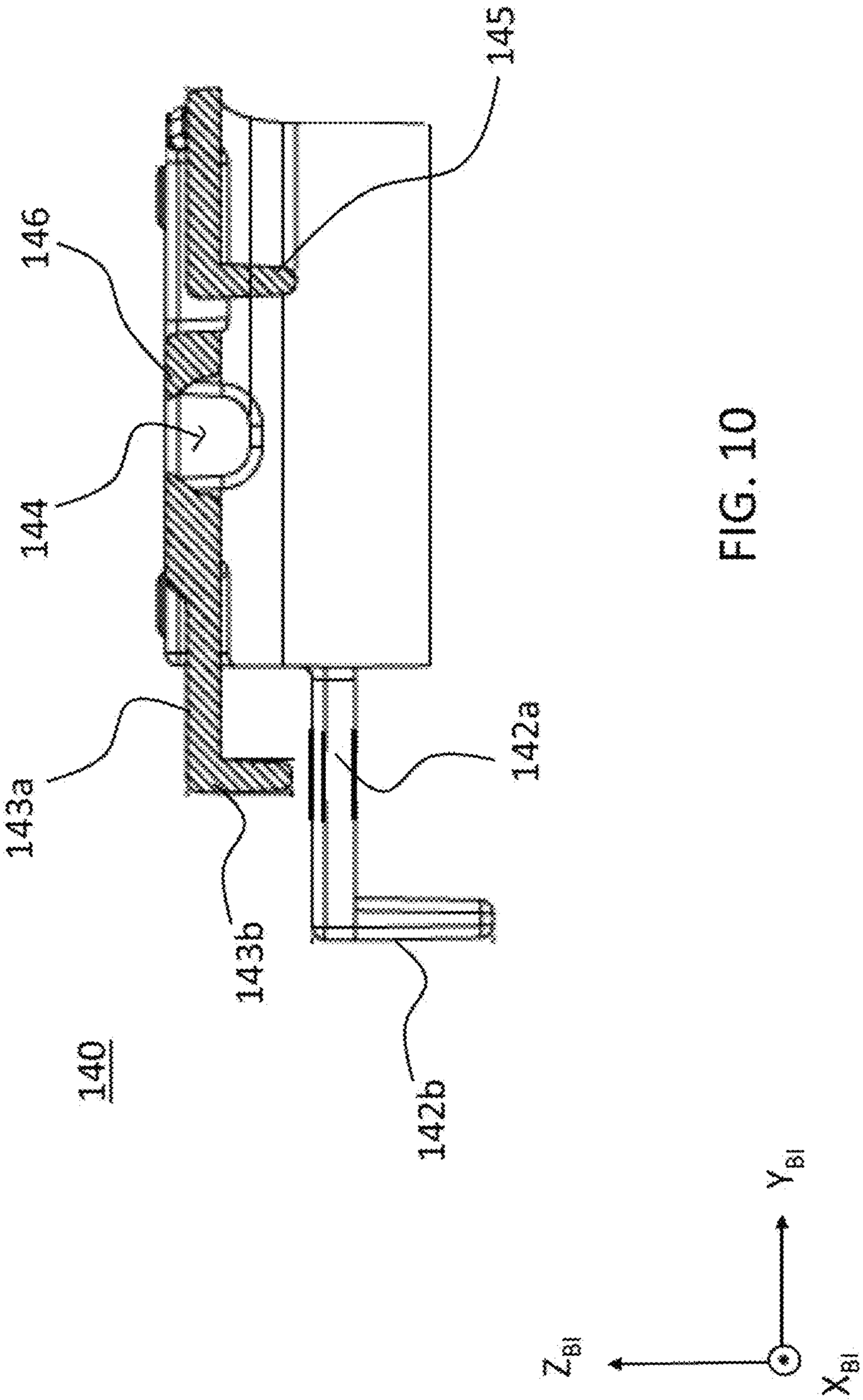
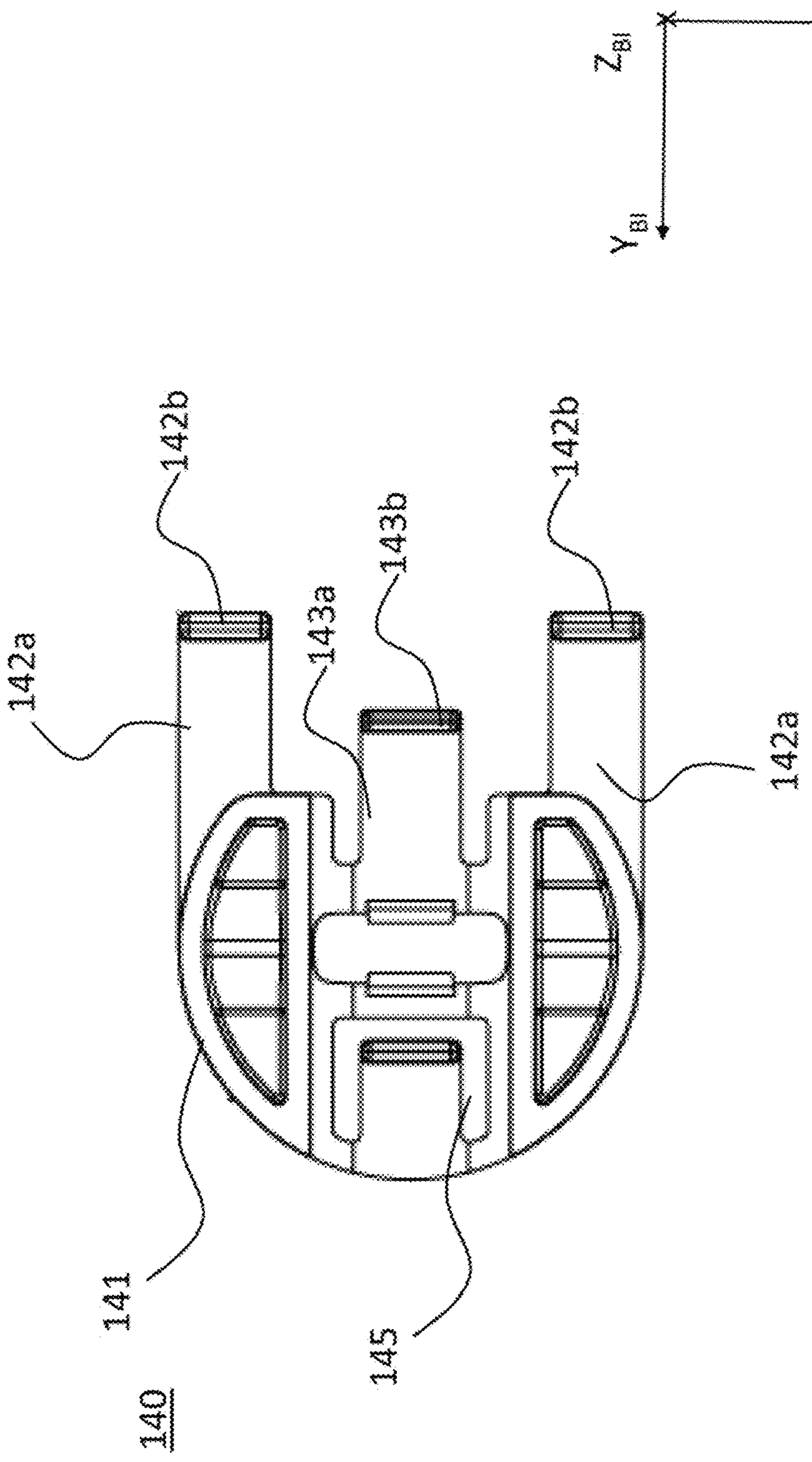


FIG. 10

FIG. 11



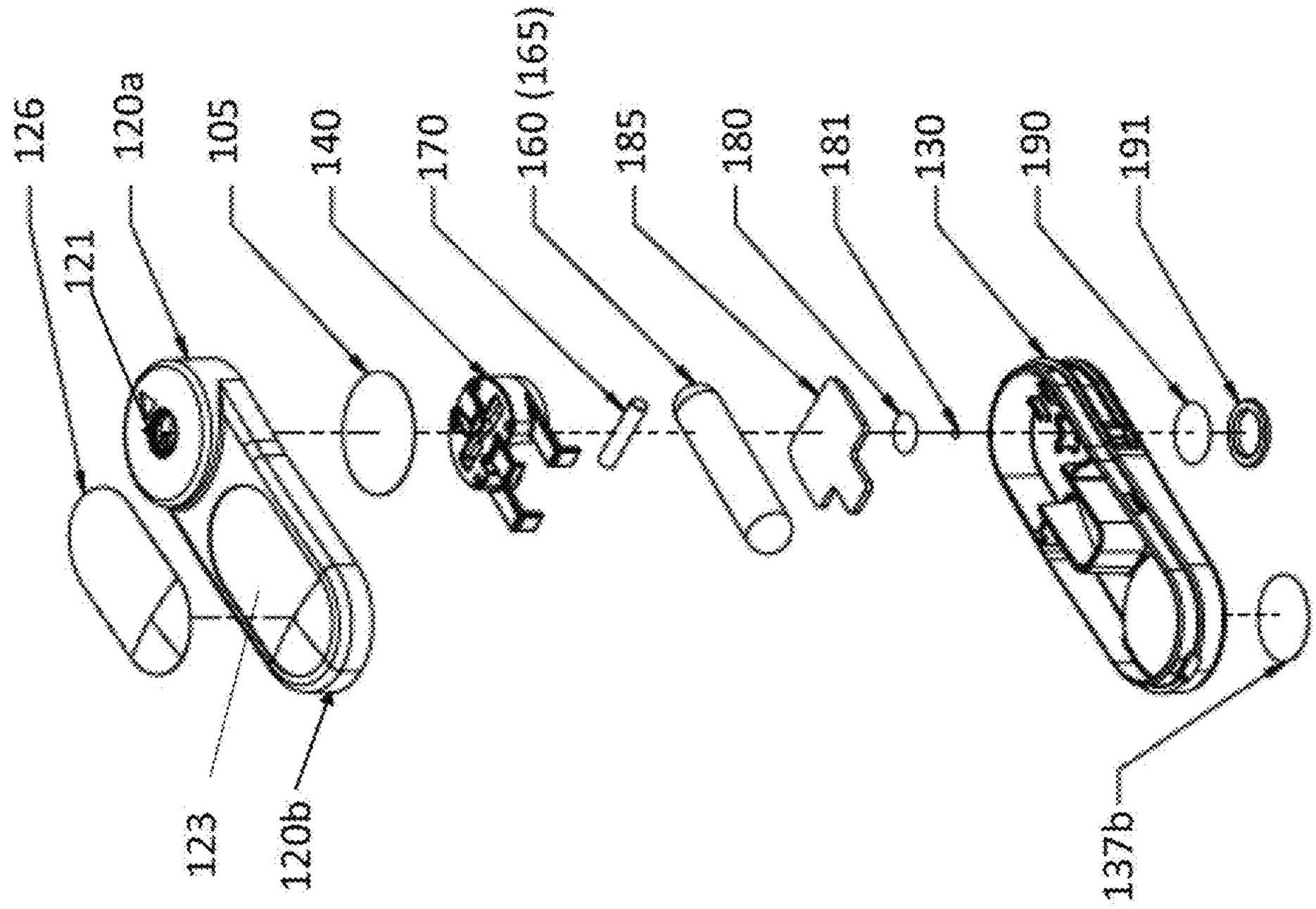
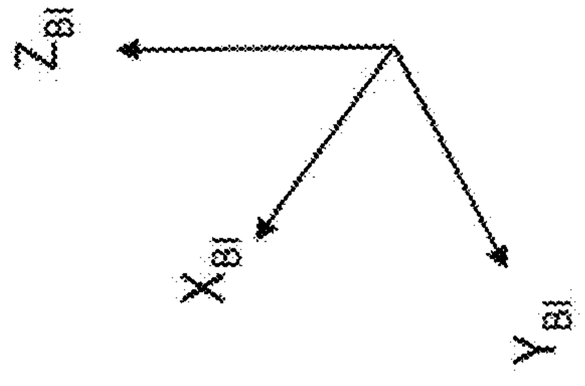

126
120a
105
140
170
160 (165)
185
180
181
130
190
191
121
123
120b
137b
100
Y_{BI}
X_{BI}
Z_{BI}

FIG. 12


181Z
181X
181Y

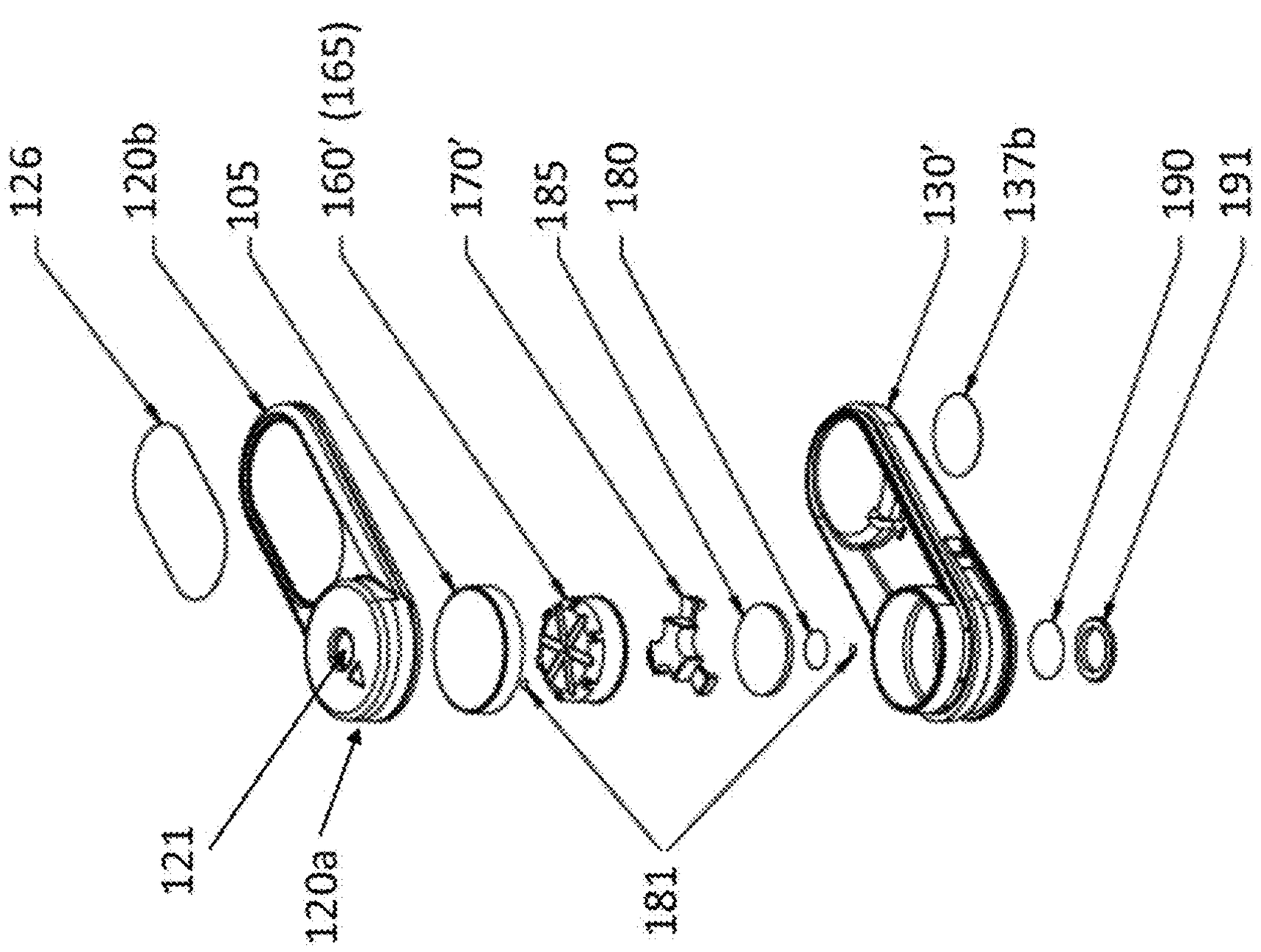

126
120b
105
160' (165)
170'
185
180
130'
137b
190
191
121
120a
181
100'

FIG. 13

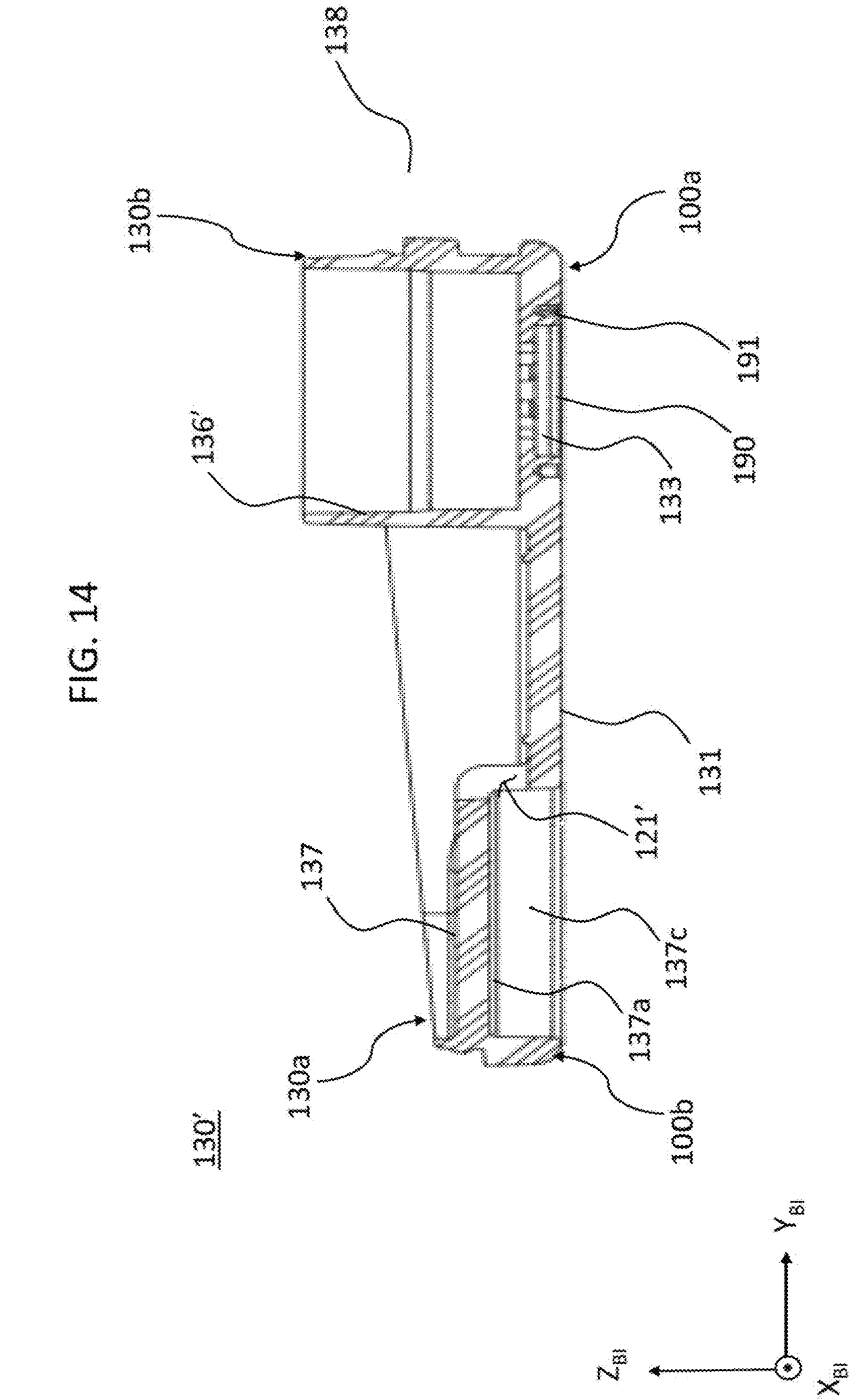
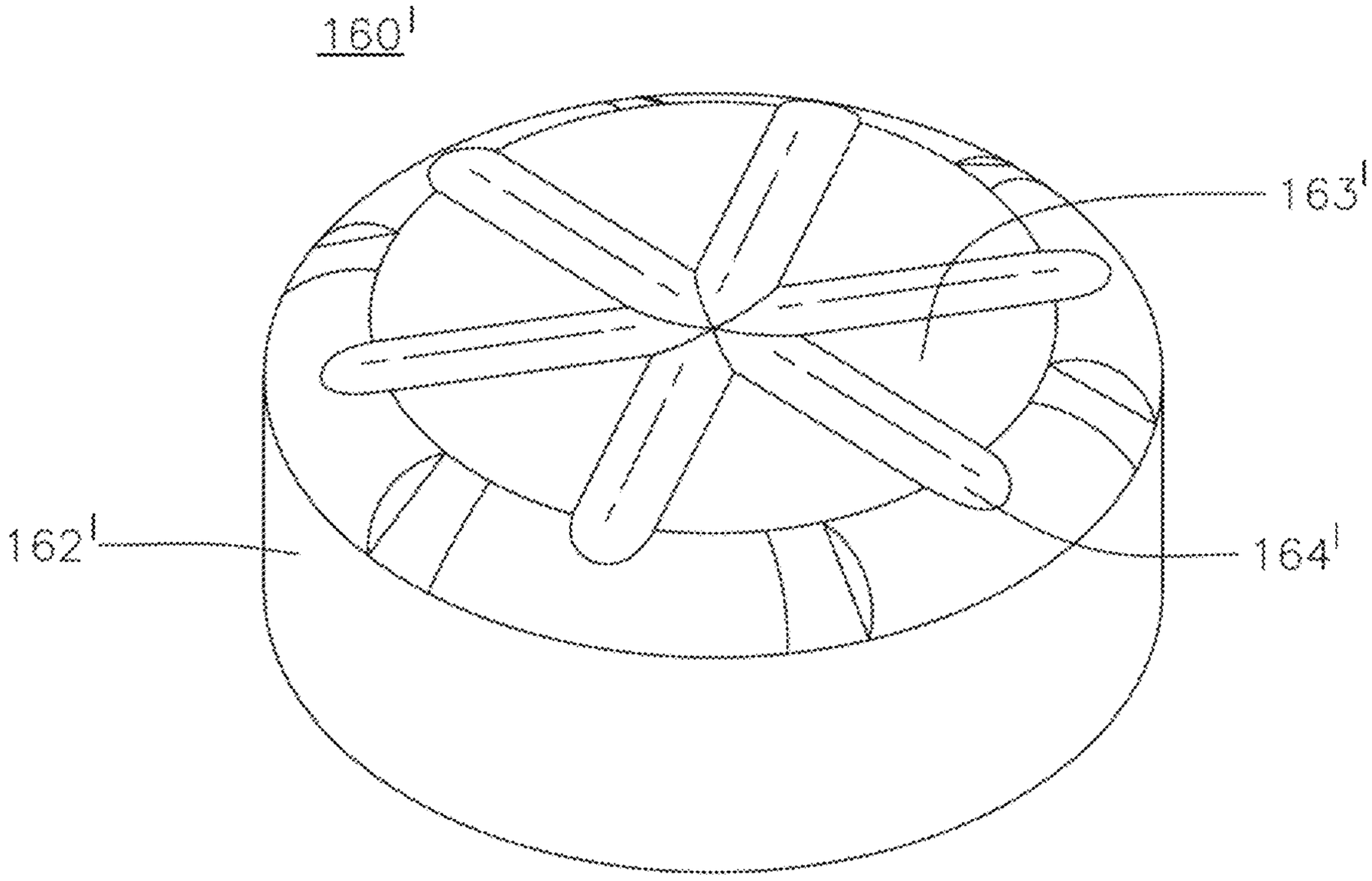

138
130b
100a
191
136'
190
133
131
121'
137
137c
137a
130a
100b
130'
Z_{BI}
Y_{BI}
X_{BI}

FIG. 14

FIG. 15



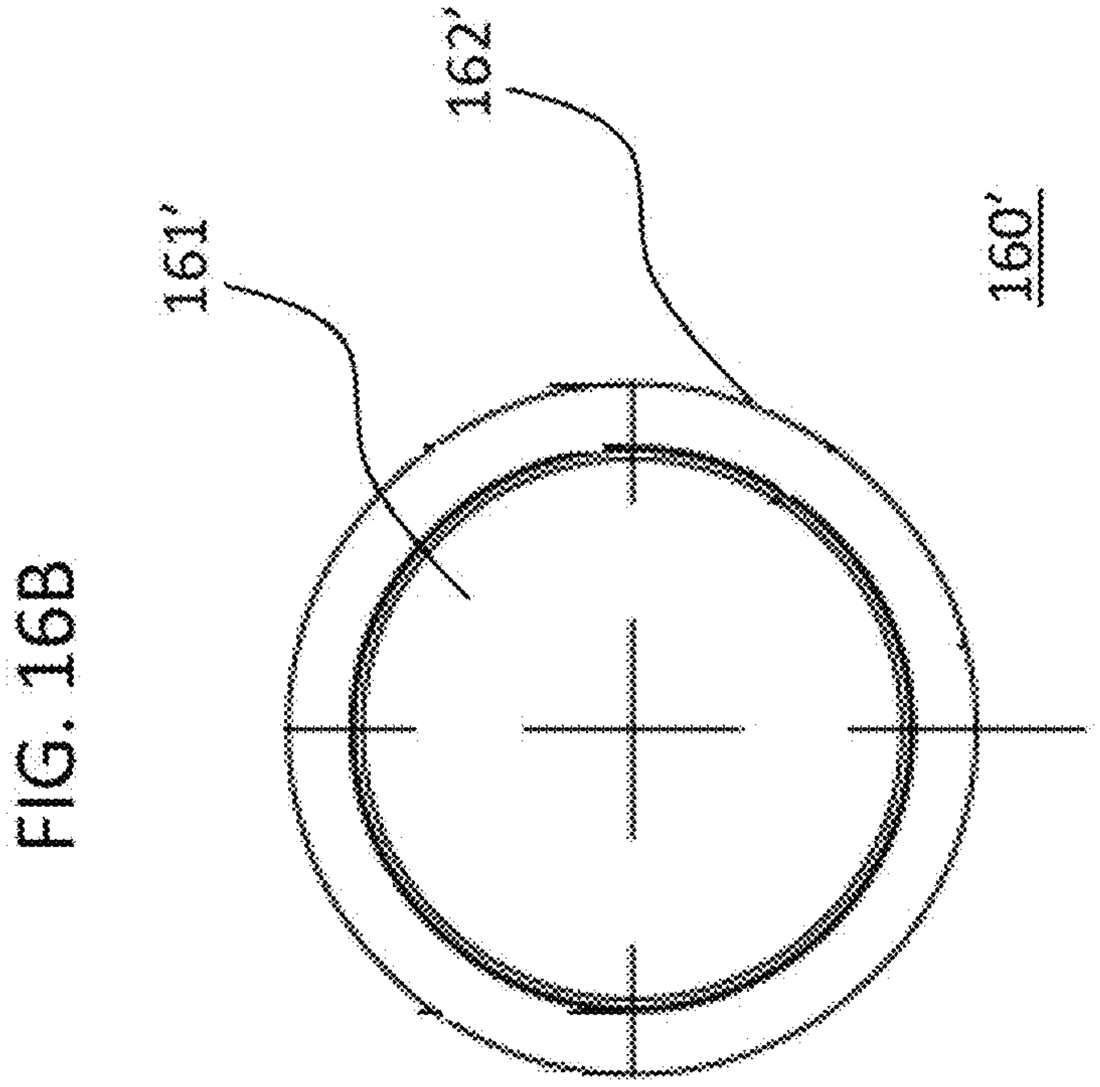


FIG. 16B

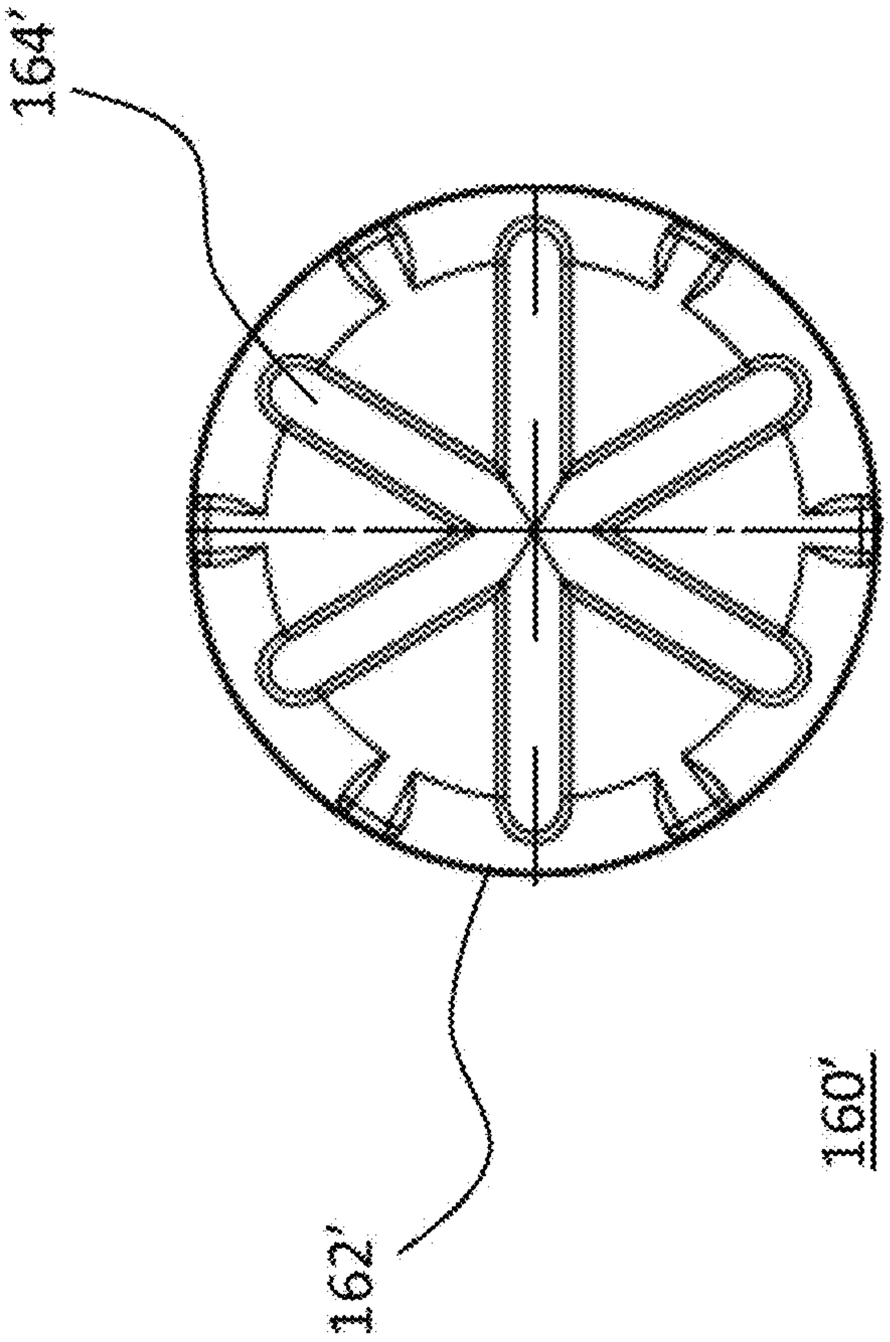


FIG. 16A

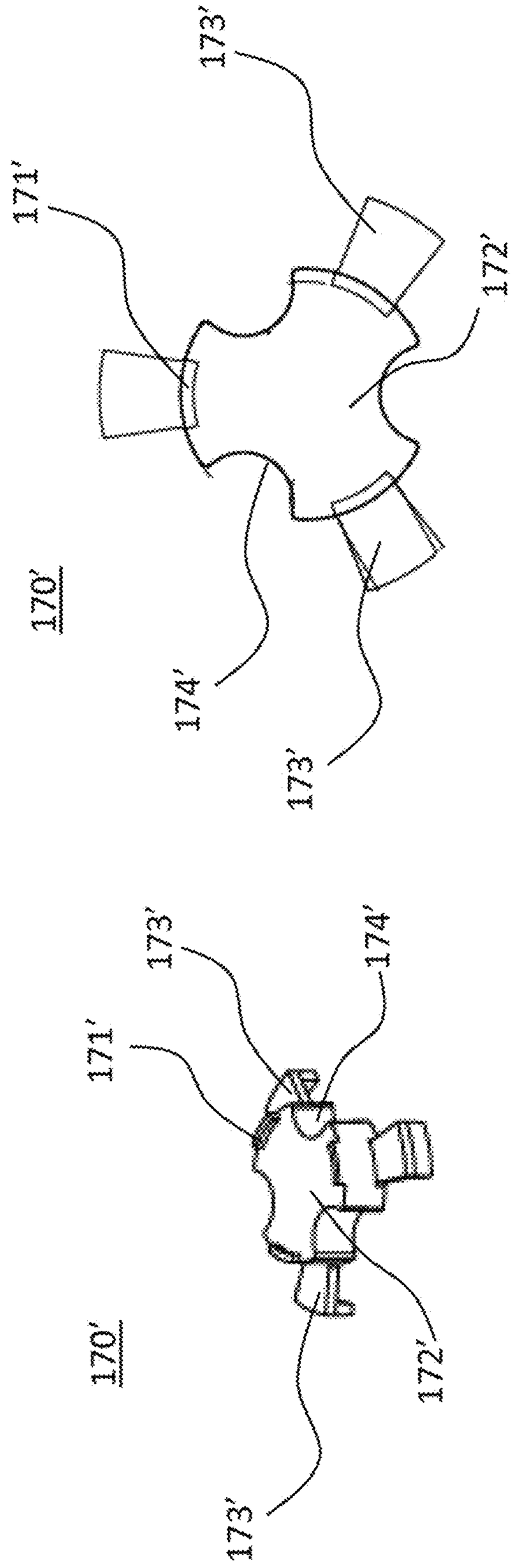
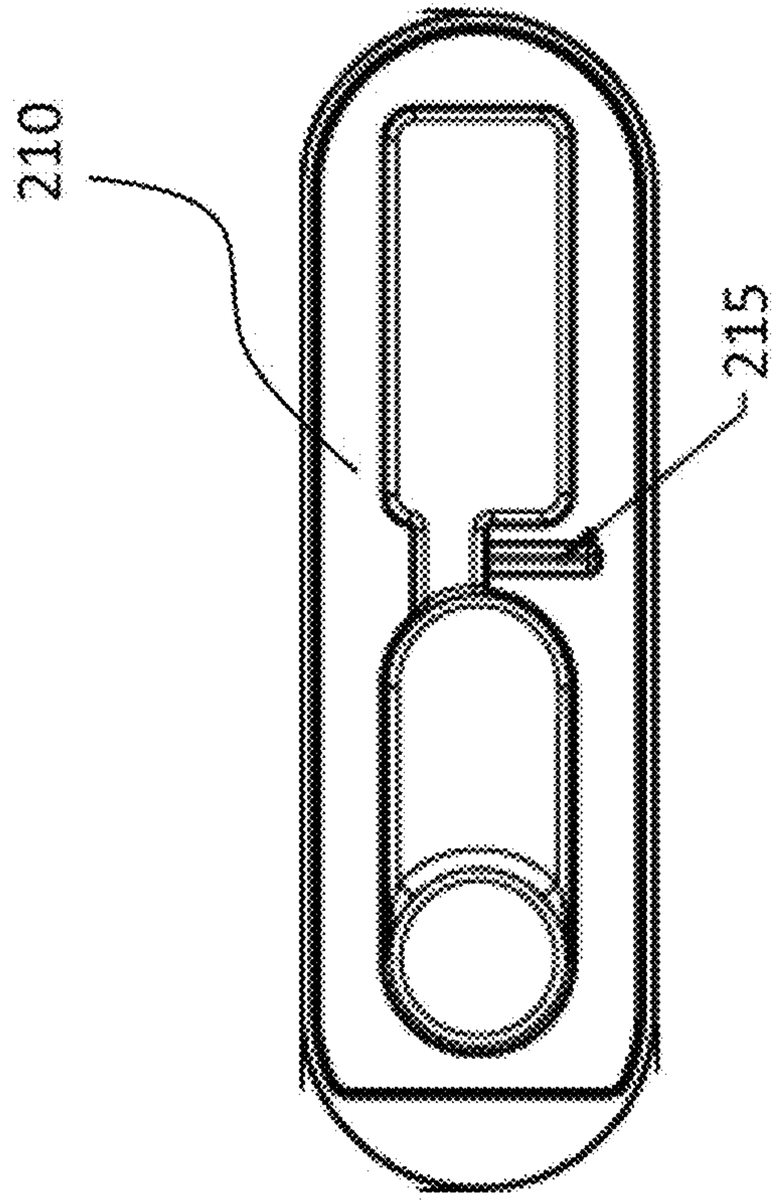


FIG. 17

FIG. 18

FIG. 19



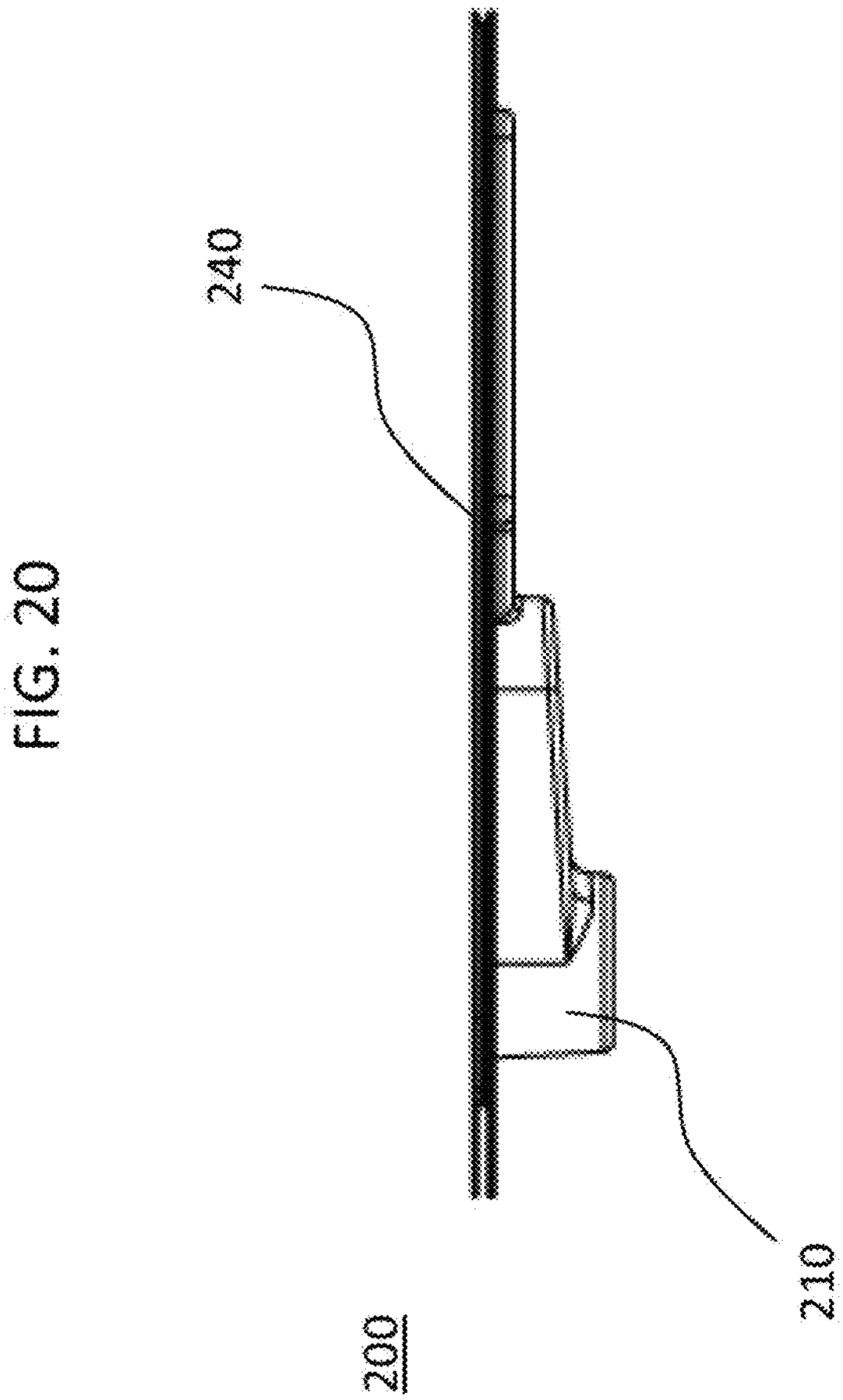


FIG. 20

FIG. 21

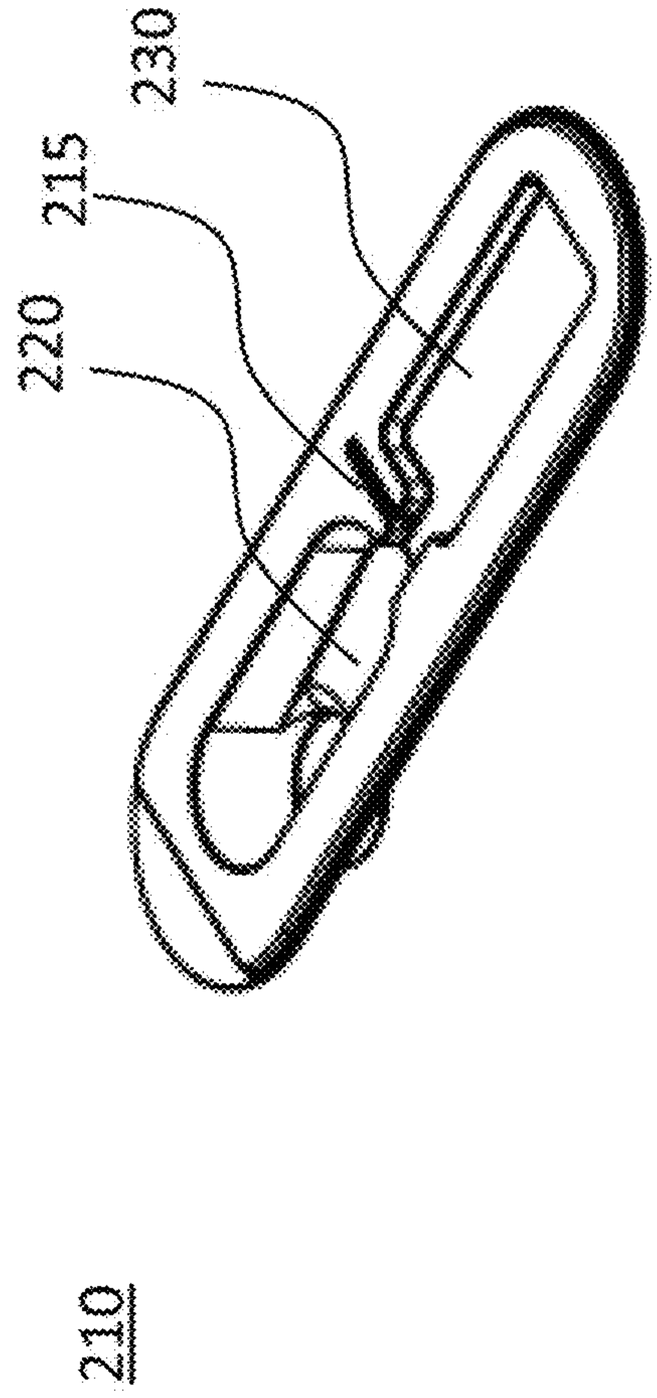


FIG. 23

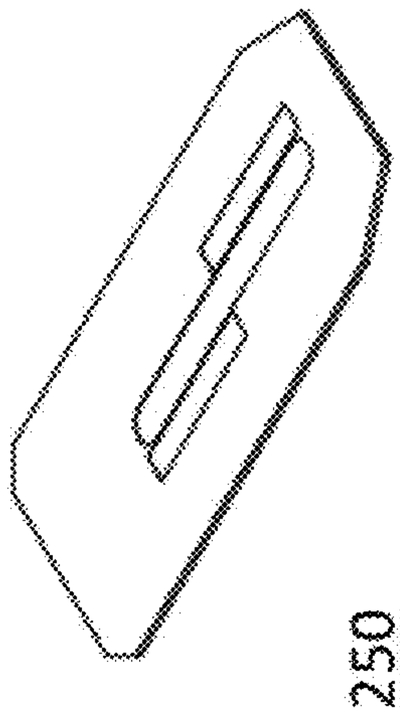
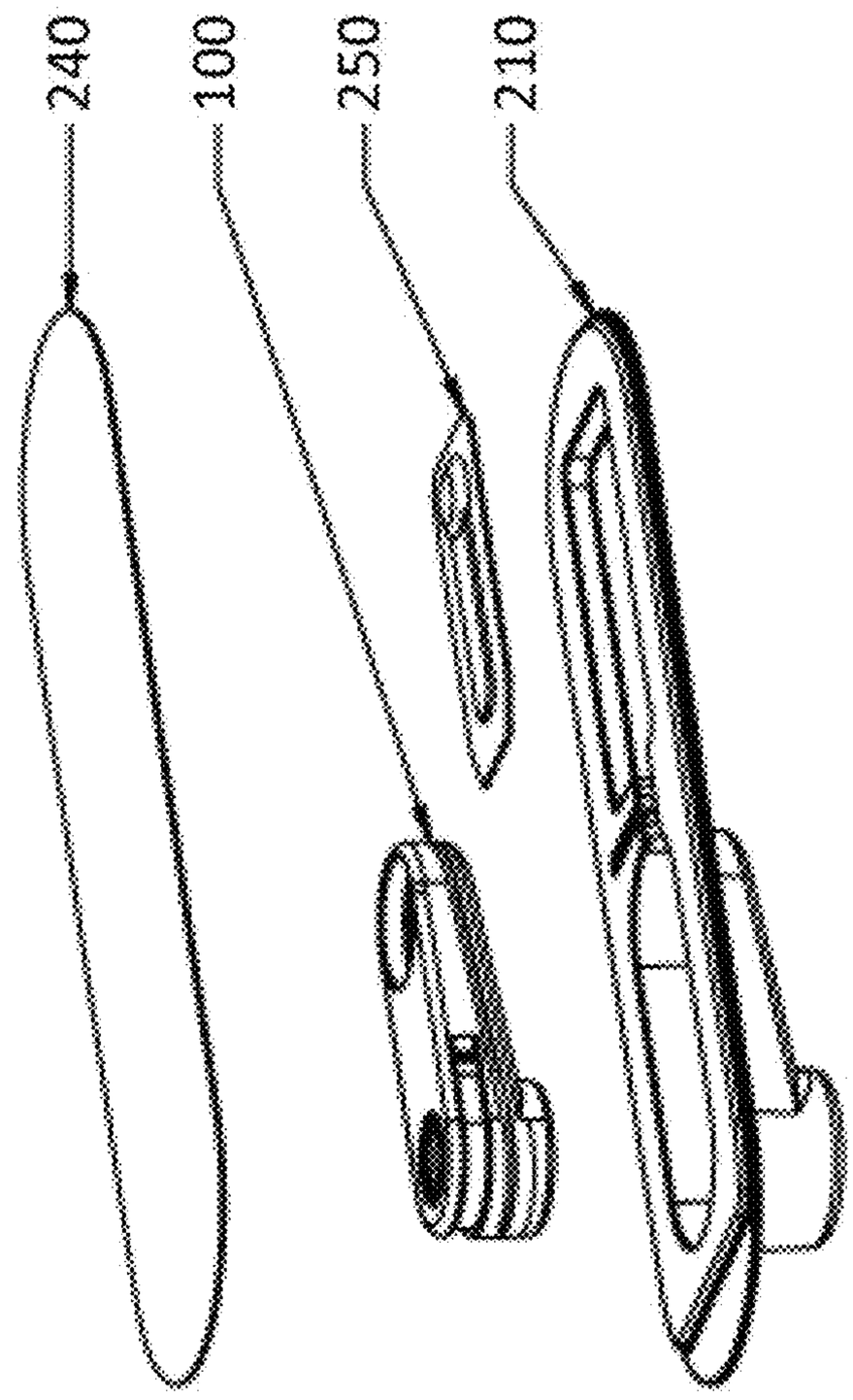


FIG. 22

FIG. 24



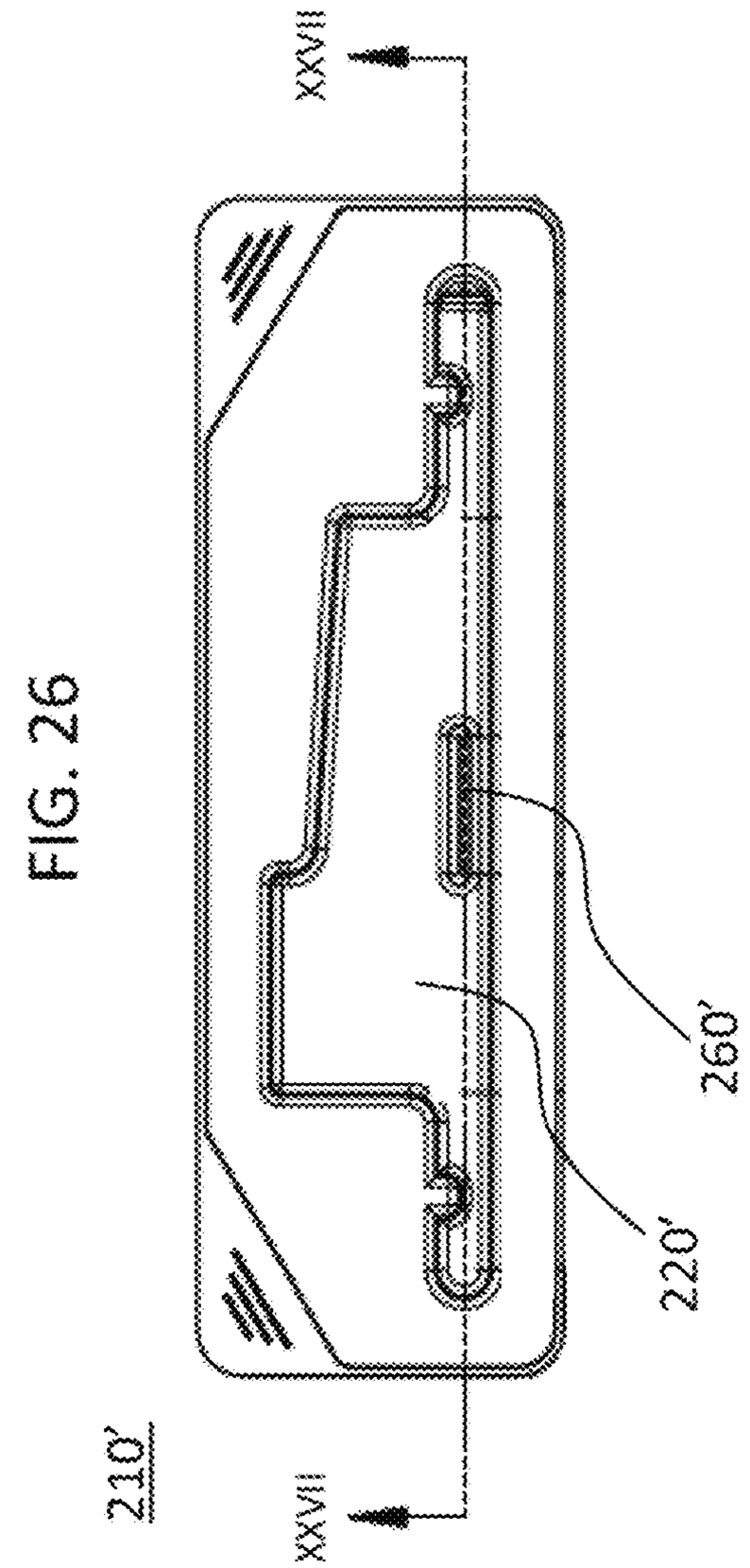


FIG. 26

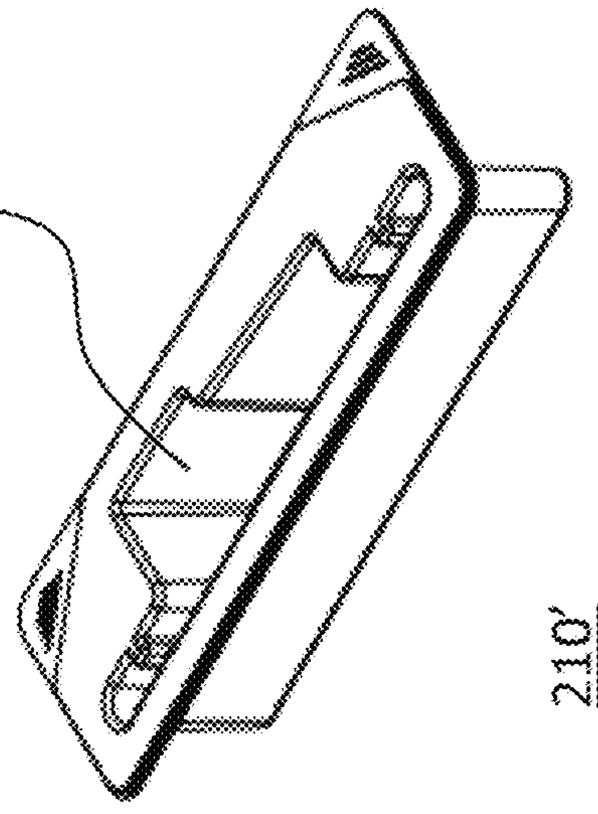


FIG. 25

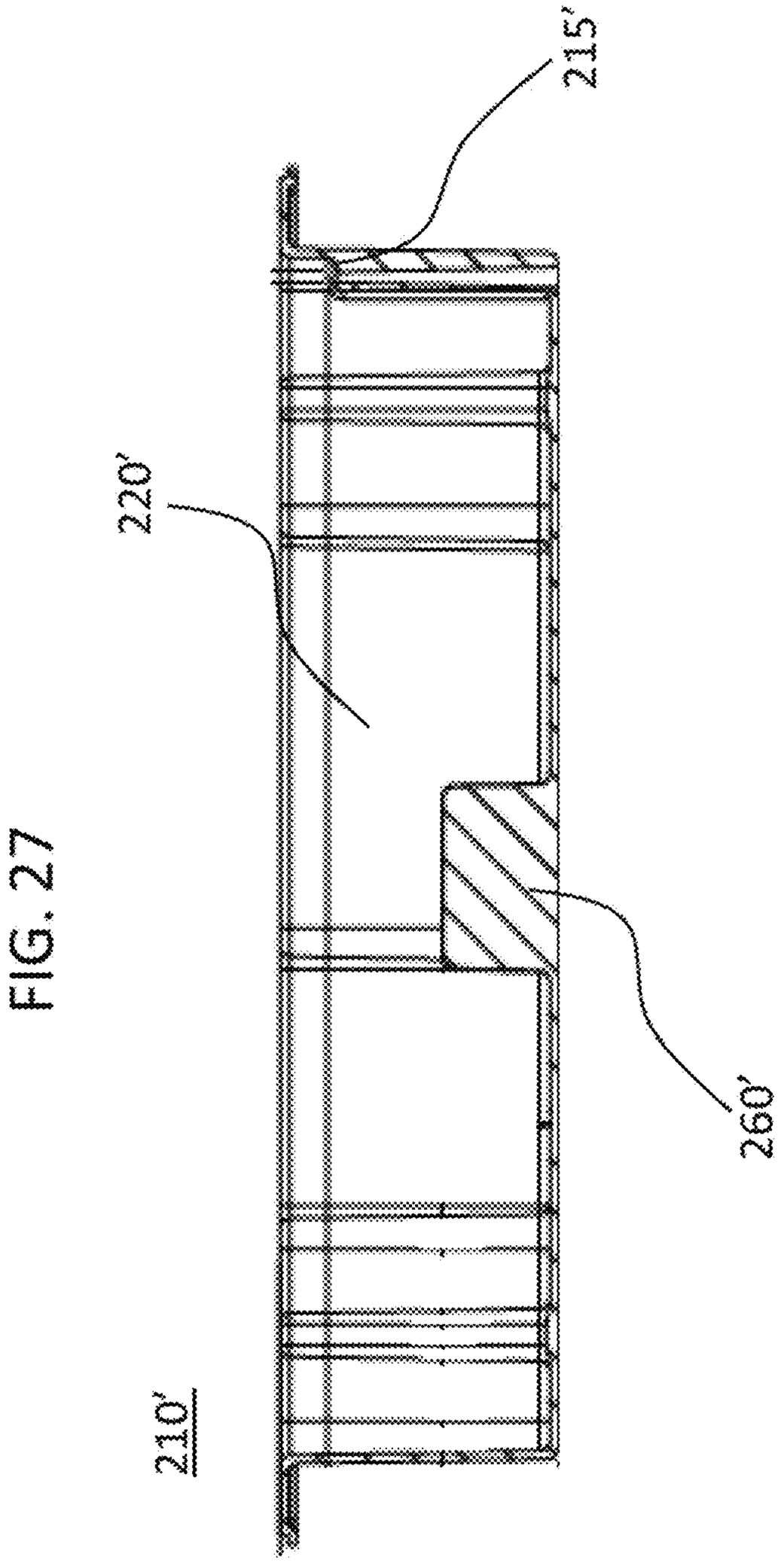


FIG. 27

FIG. 28

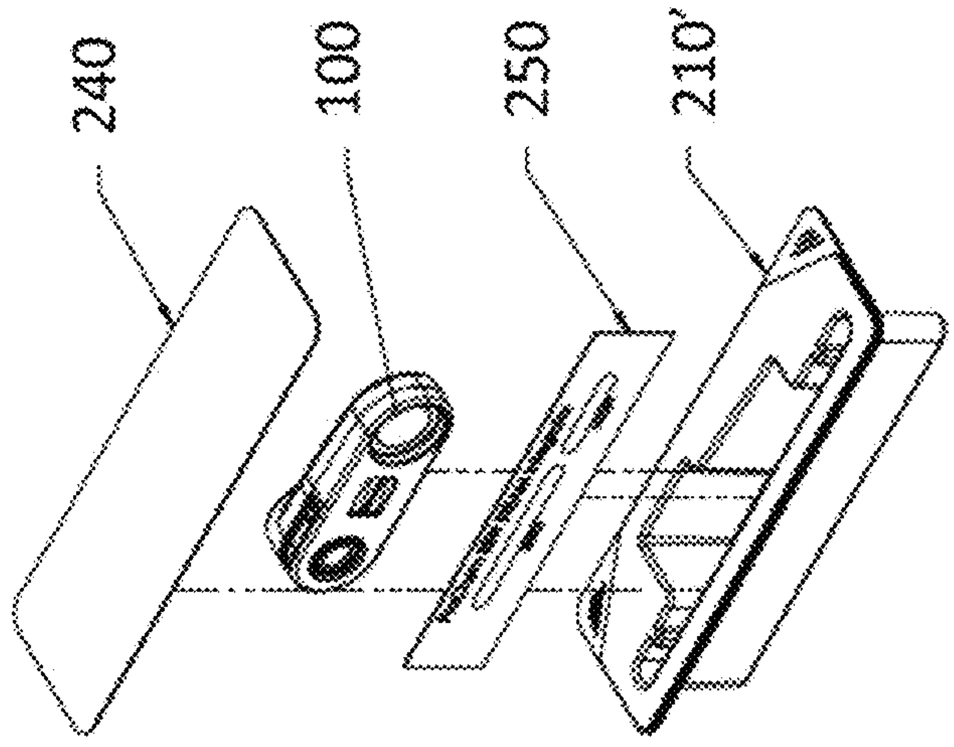
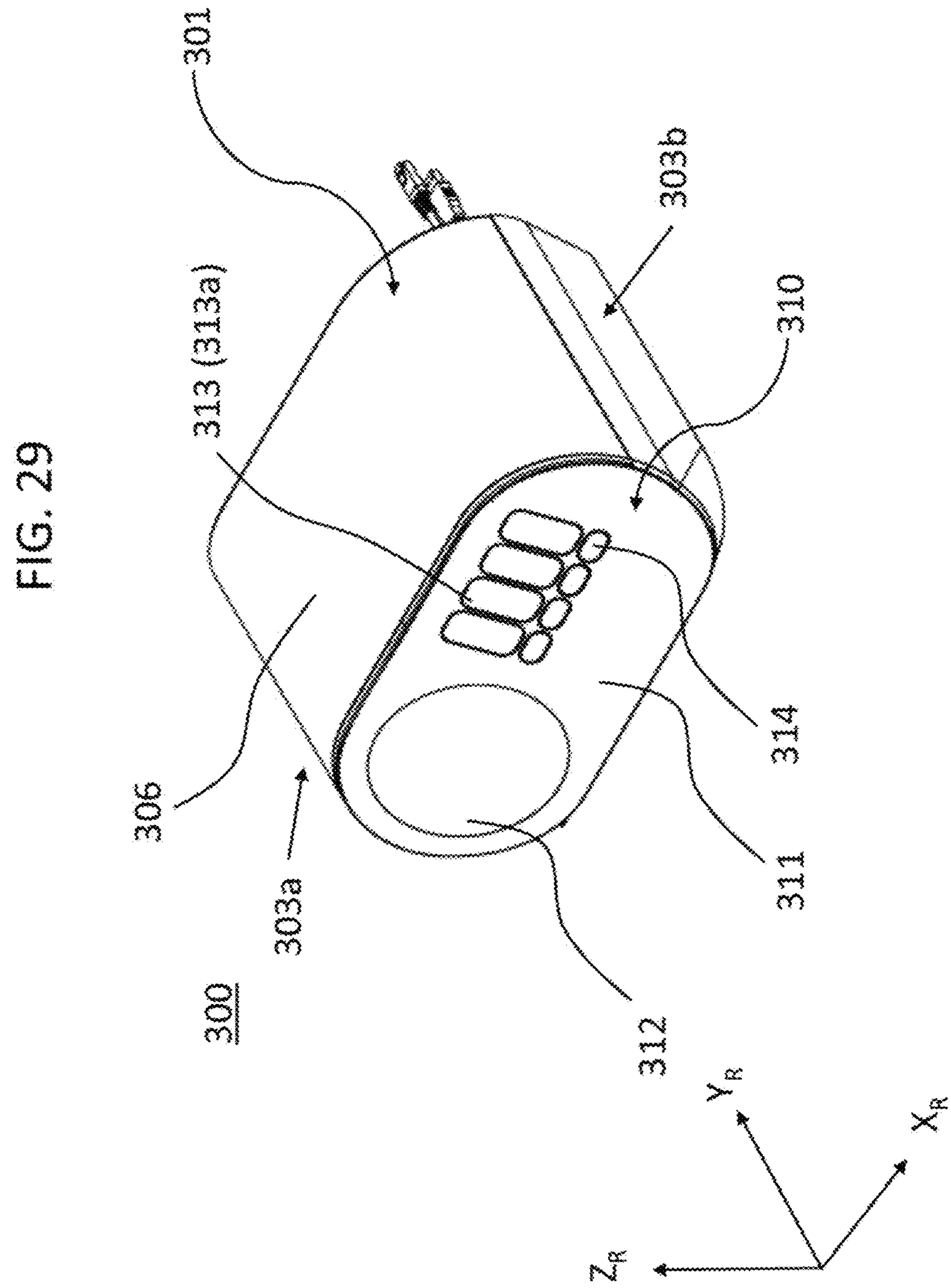


FIG. 29



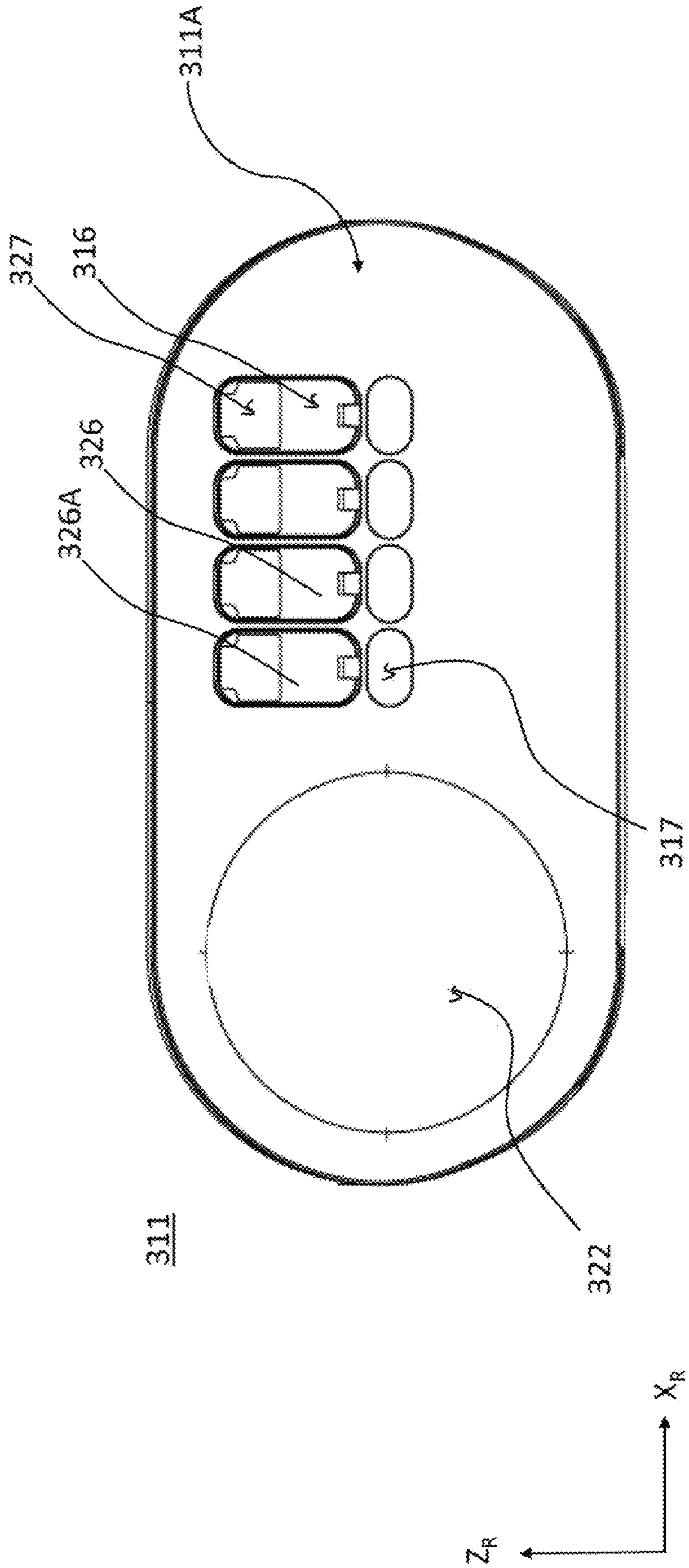
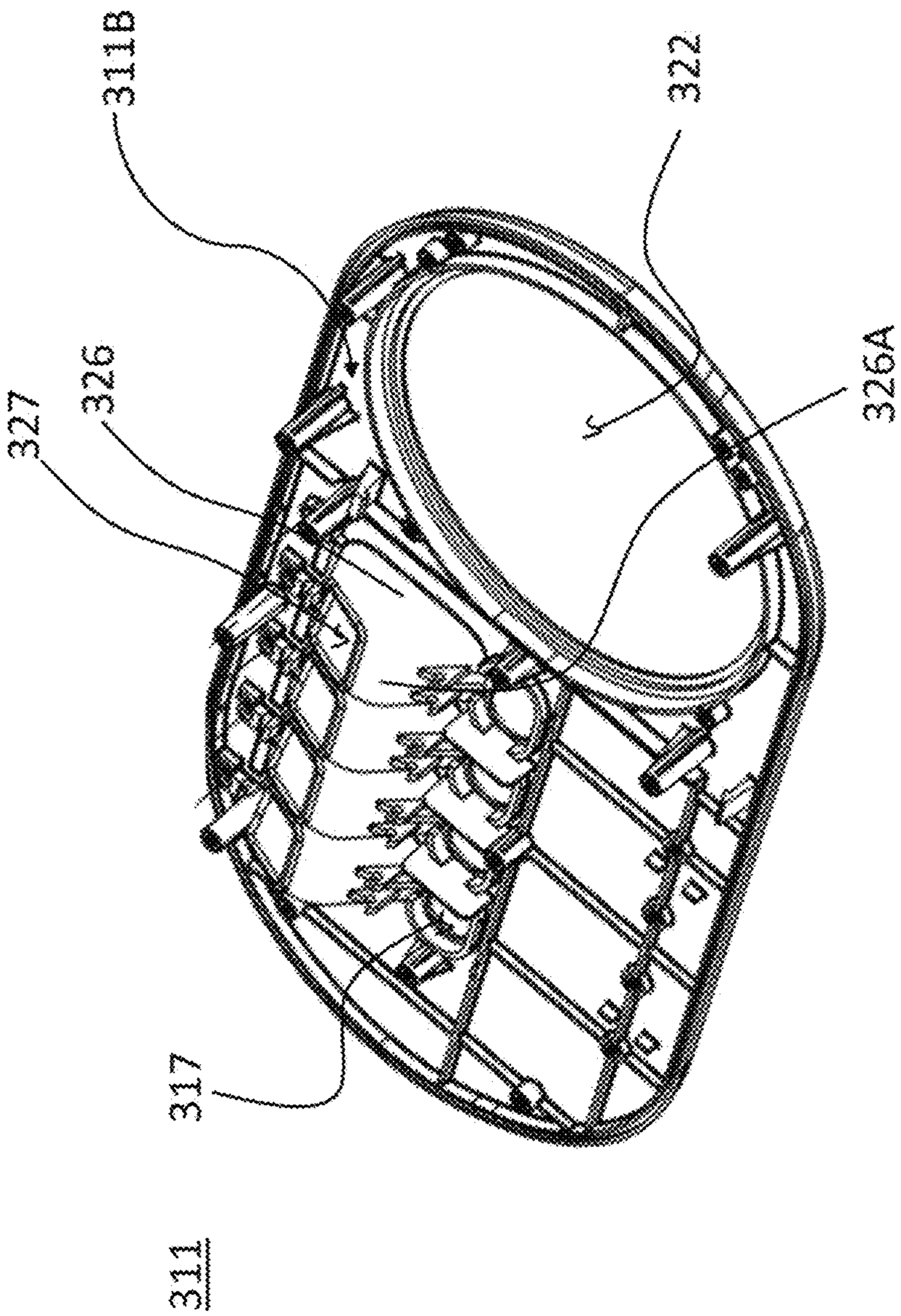


FIG. 30

FIG. 31



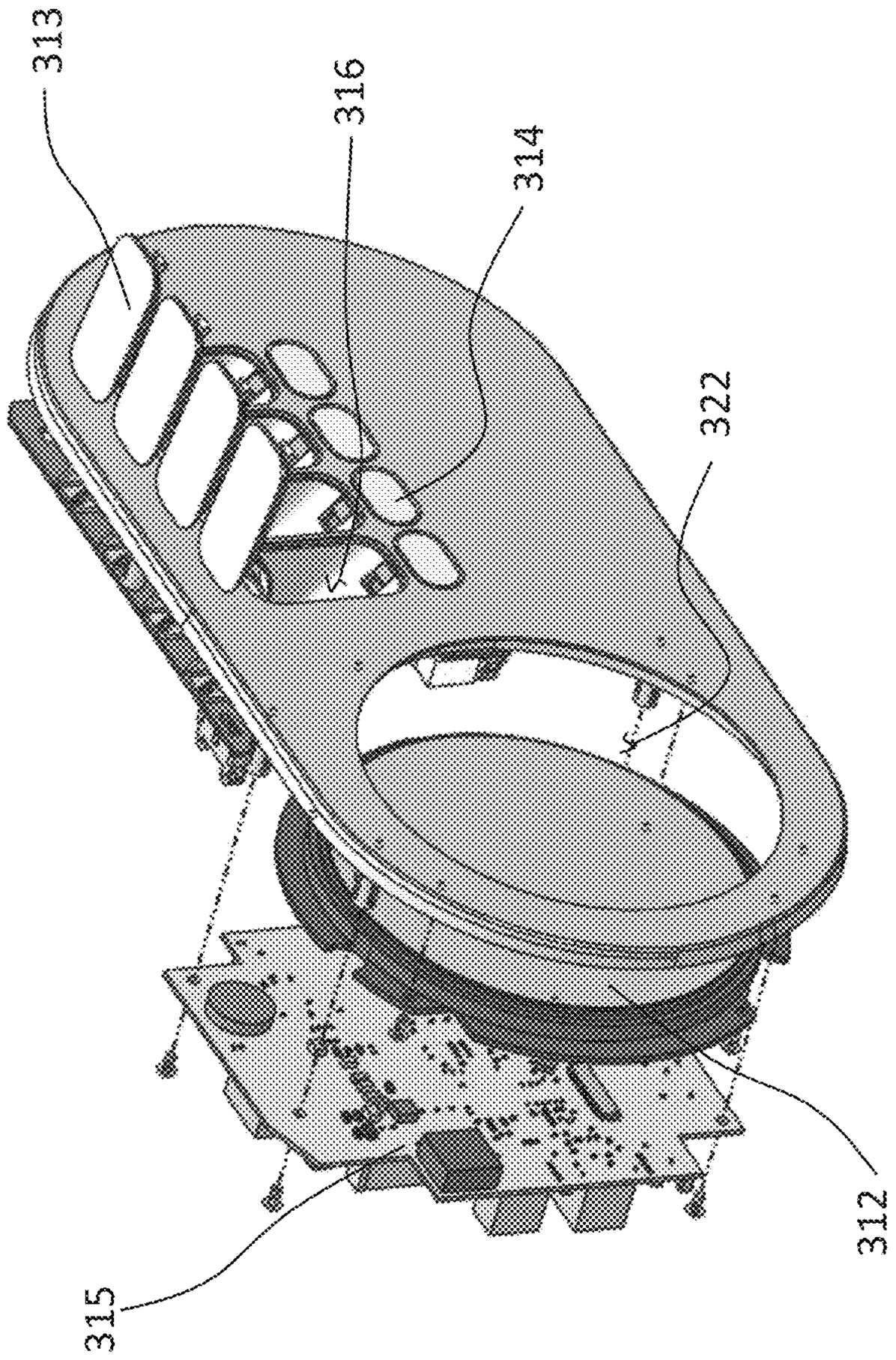


FIG. 32

FIG. 33

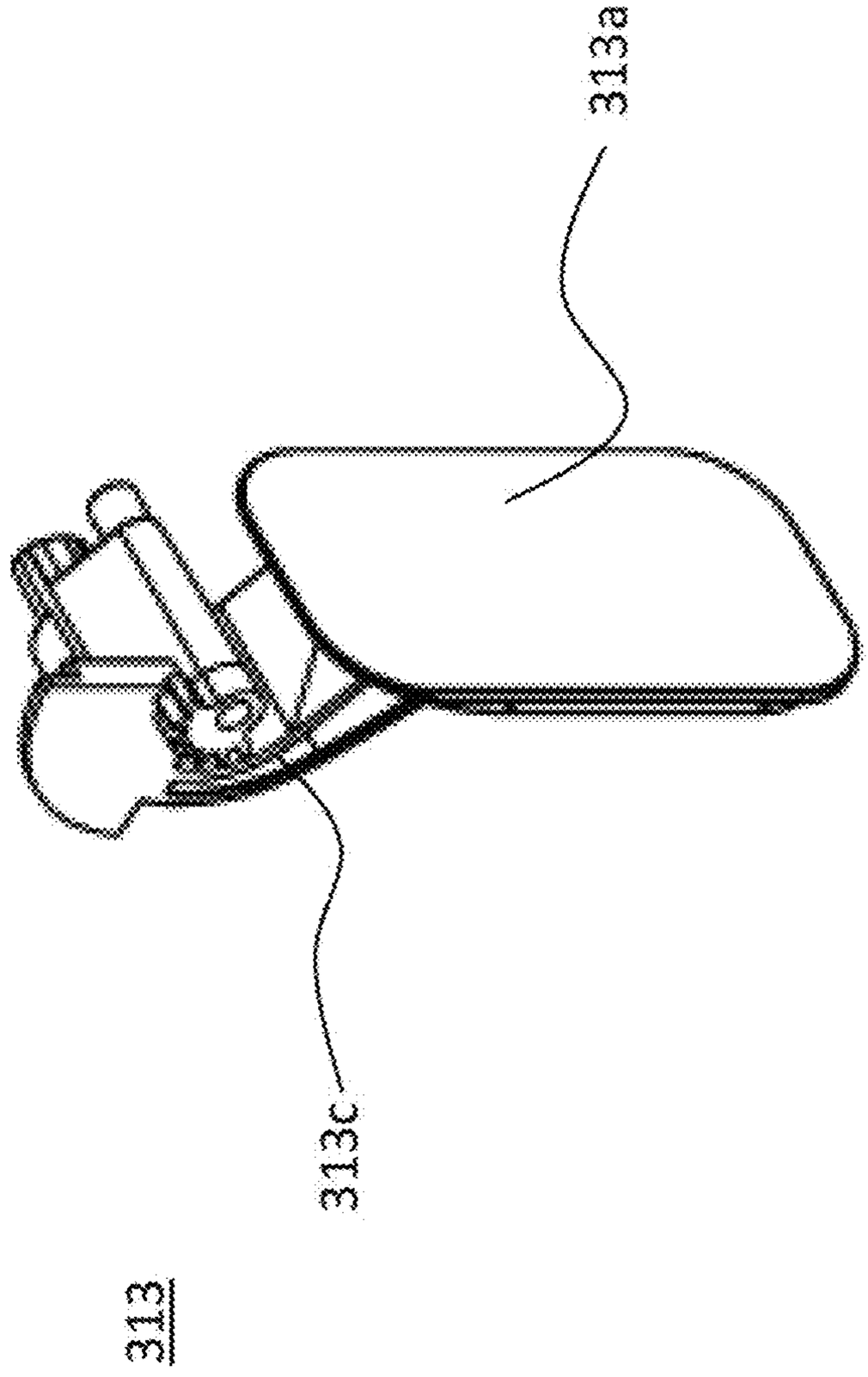
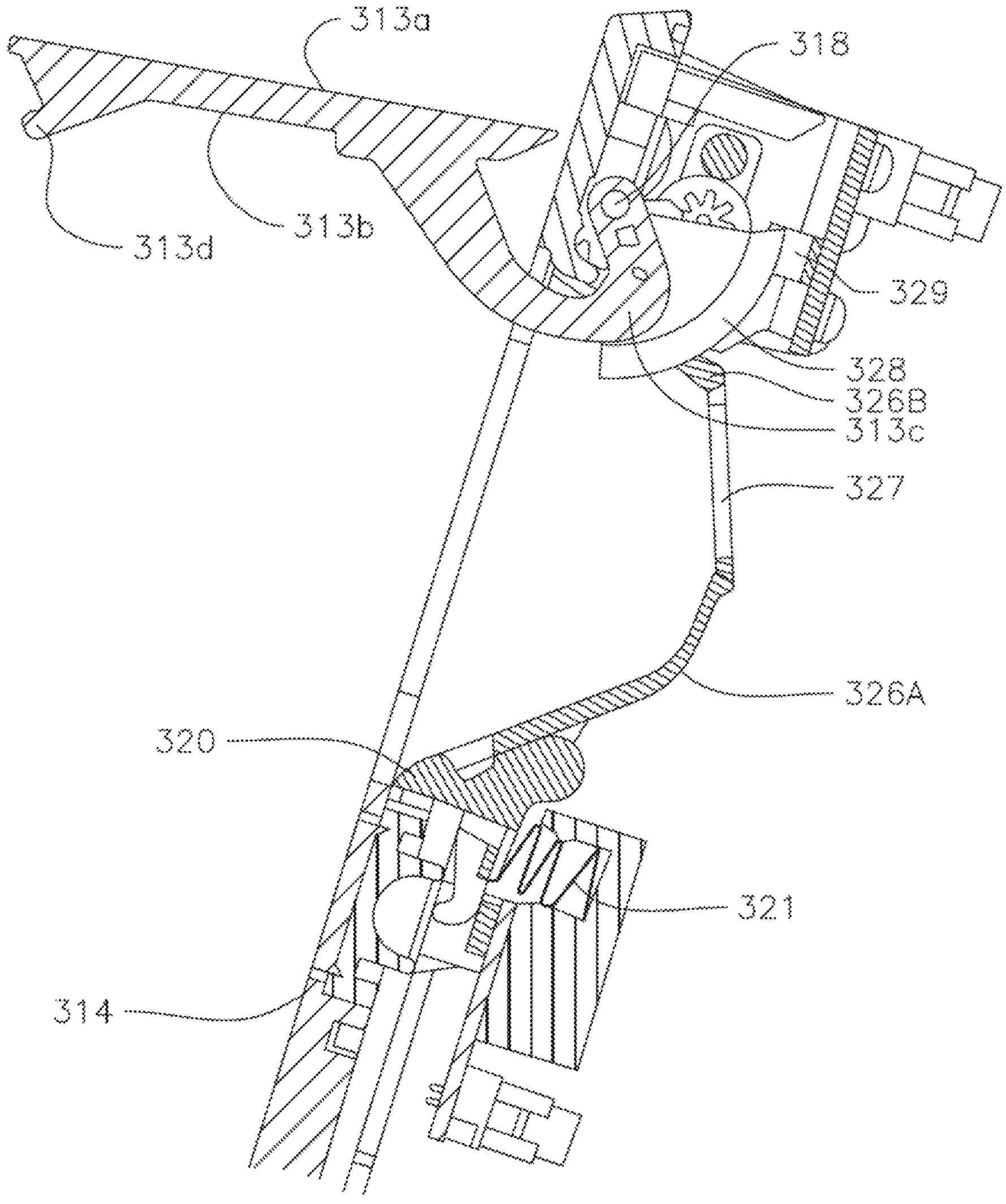


FIG. 34



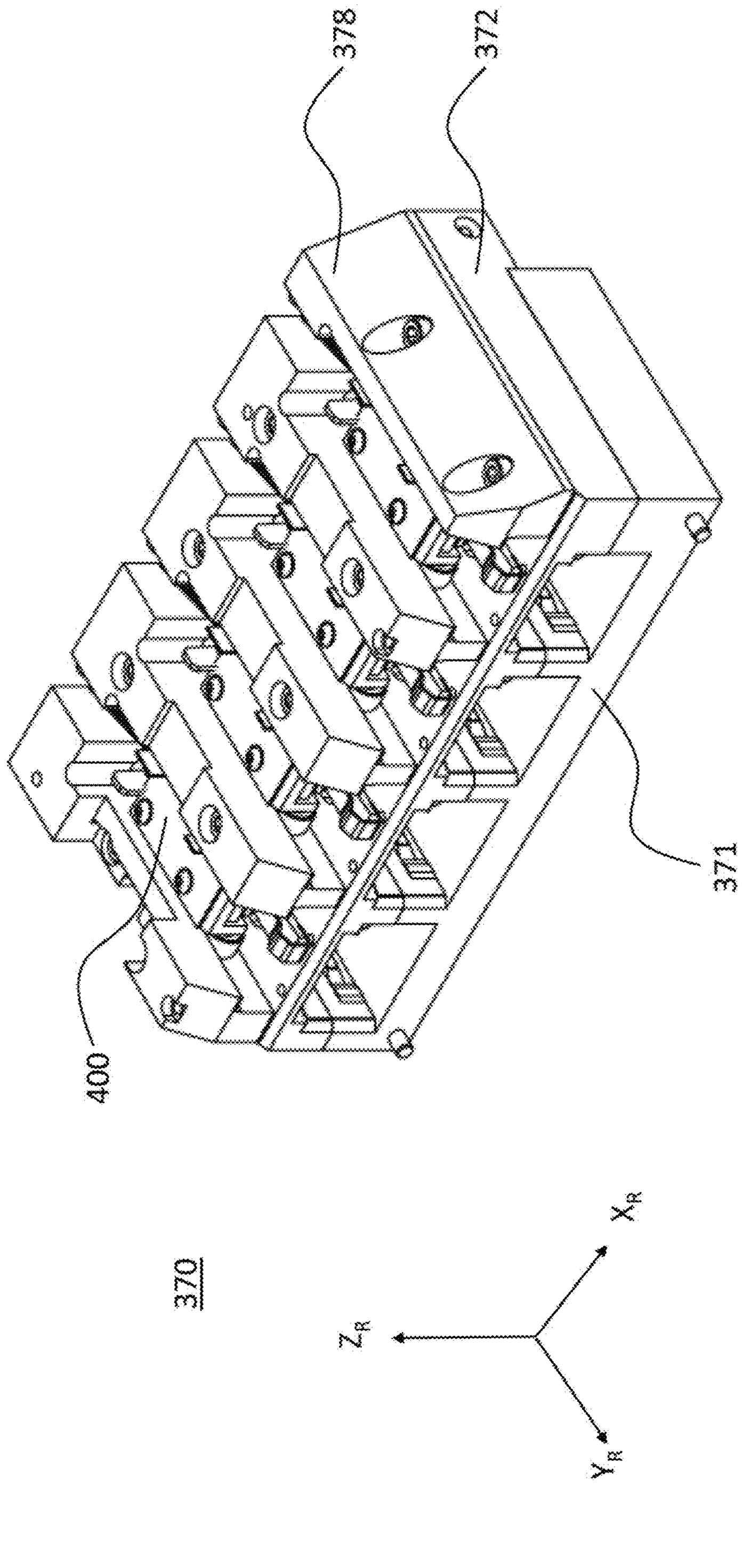


FIG. 35

FIG. 36

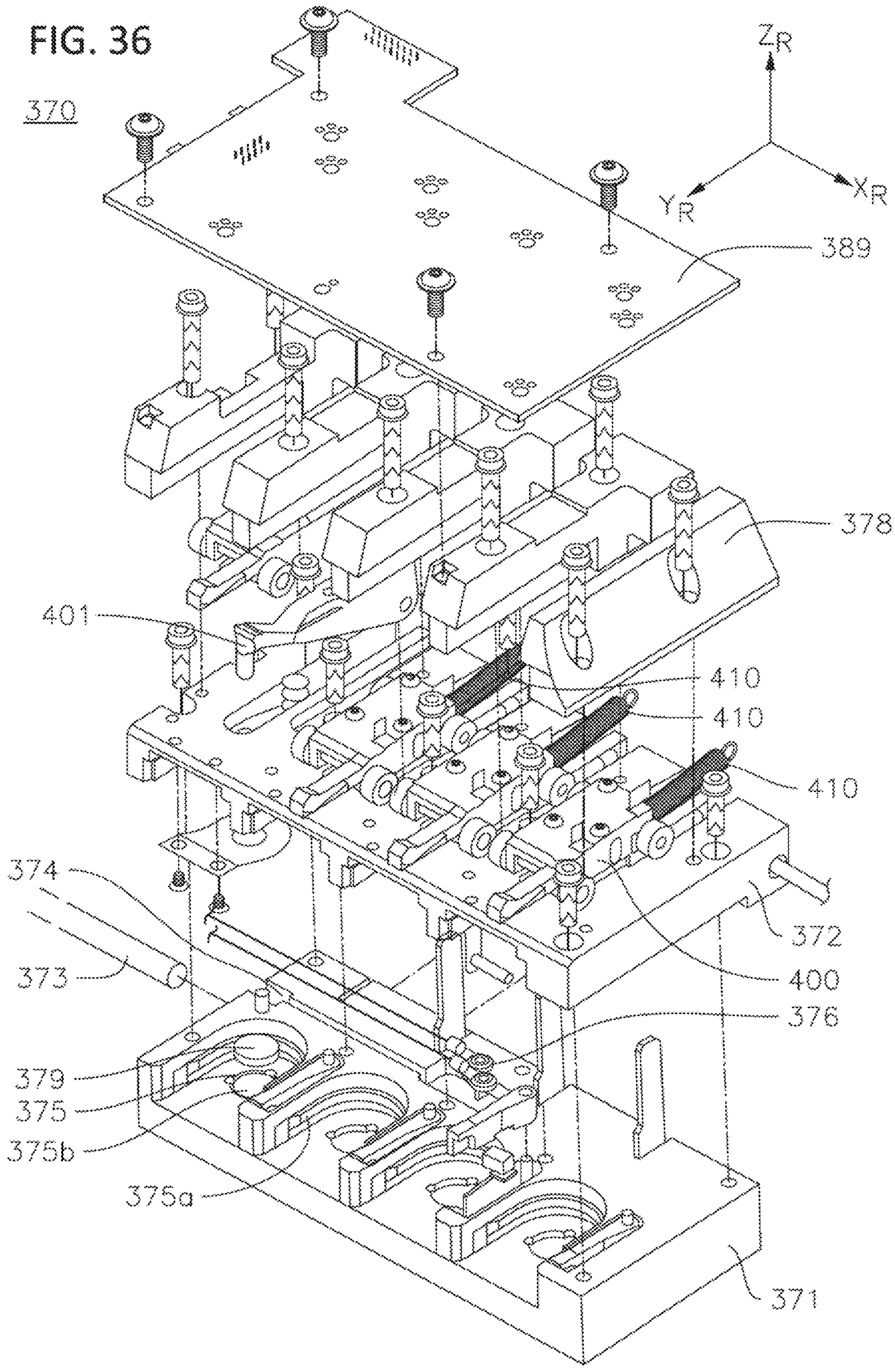

370
ZR
YR
XR
389
378
401
410
410
410
374
373
372
400
376
379
375
375b
375a
371

FIG. 37

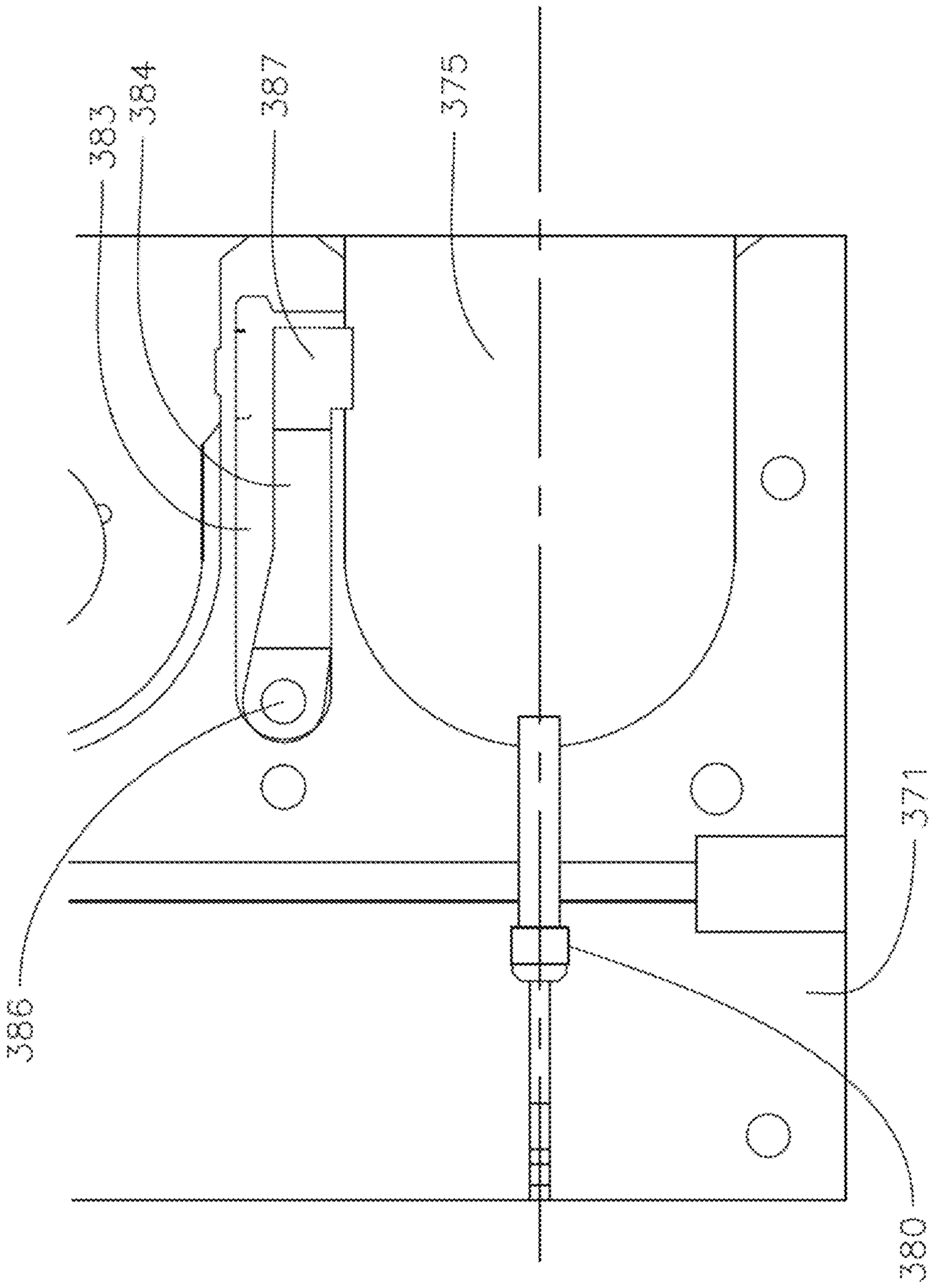
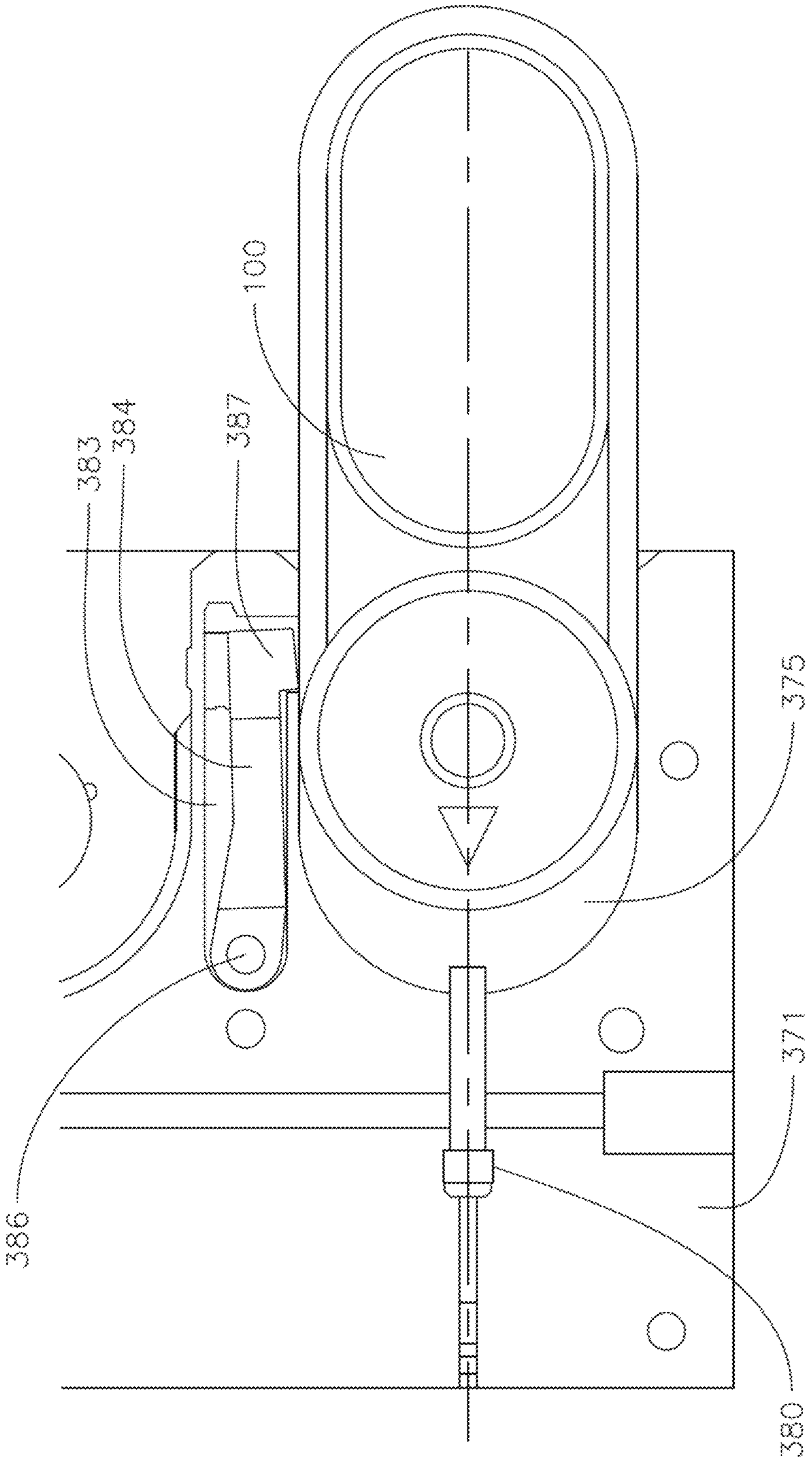


FIG. 38



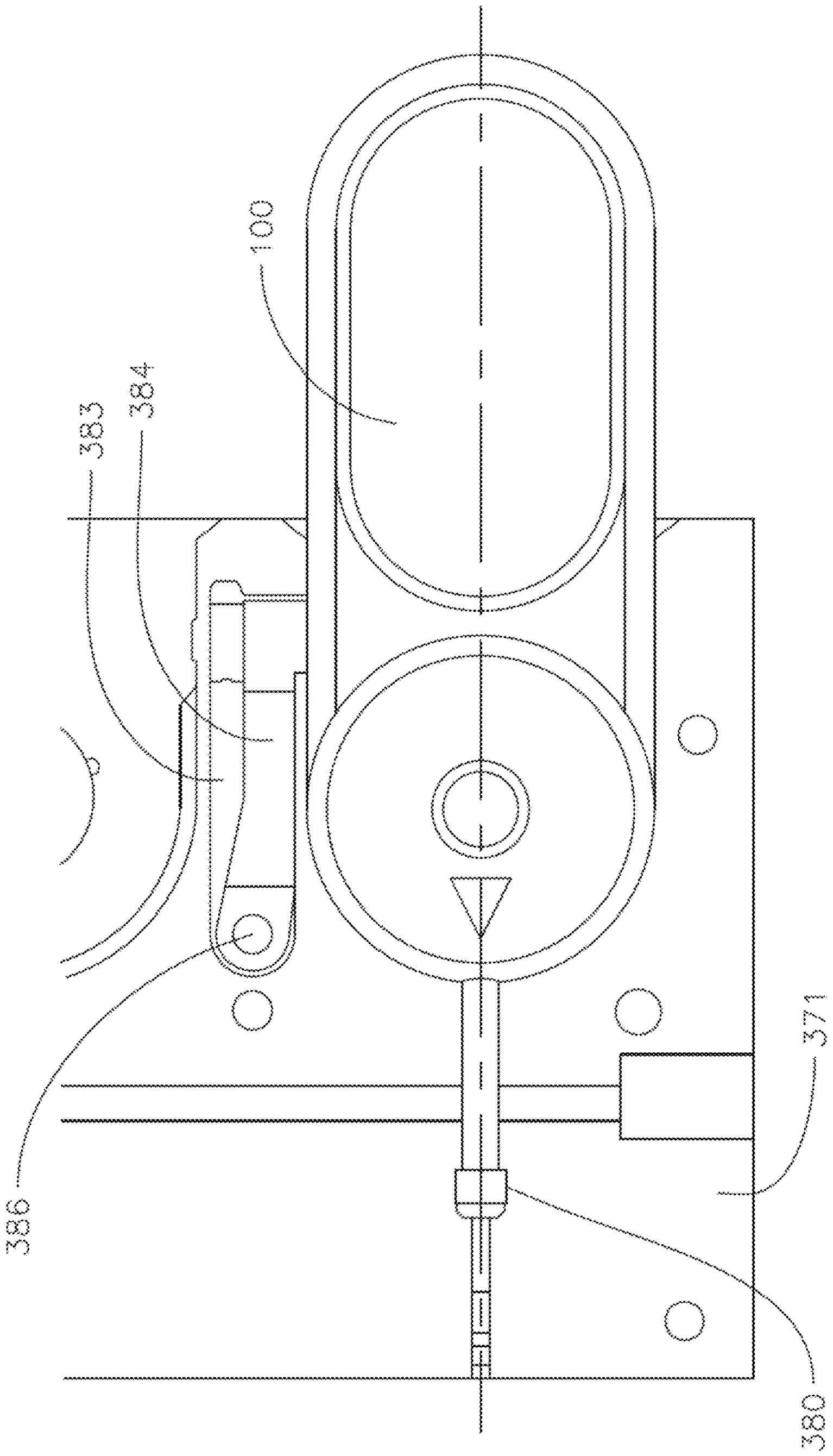


FIG. 39

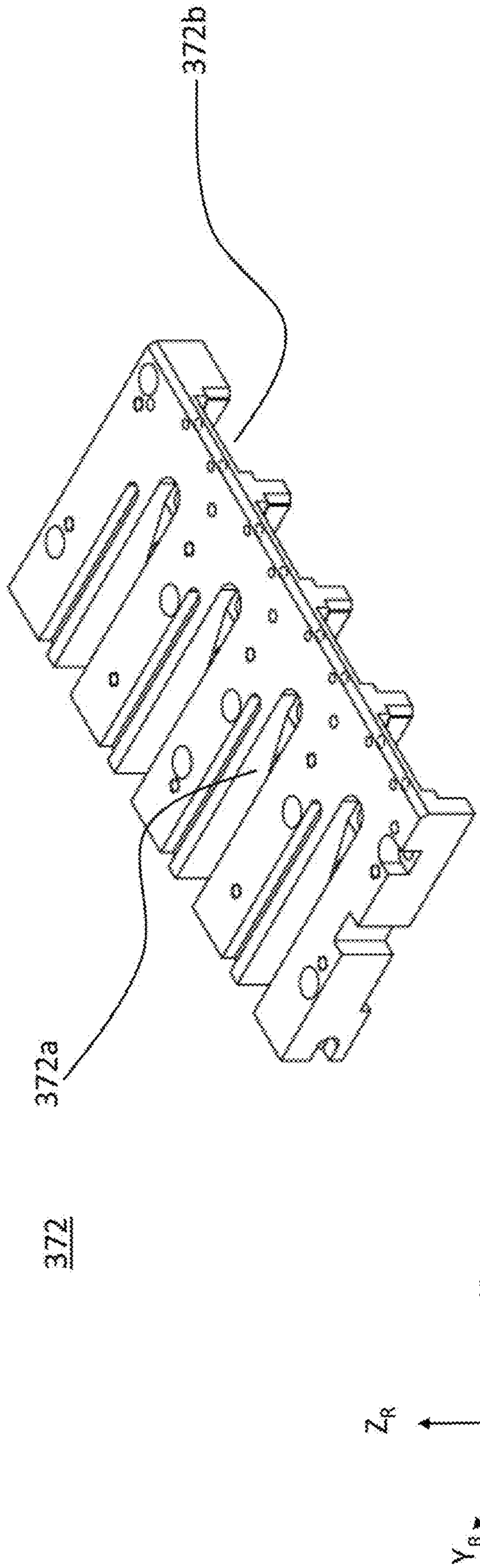


FIG. 40

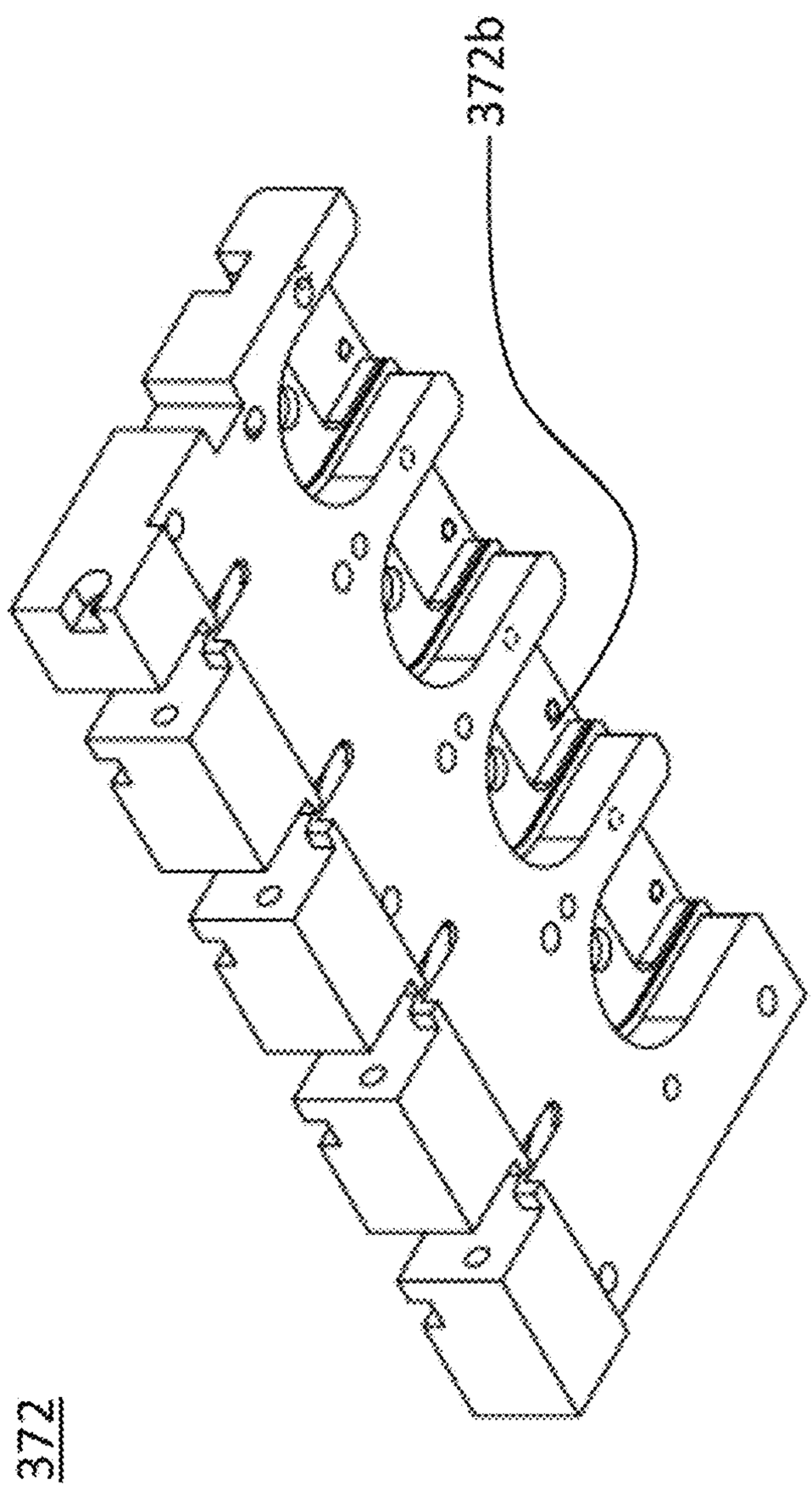


FIG. 41

FIG. 42

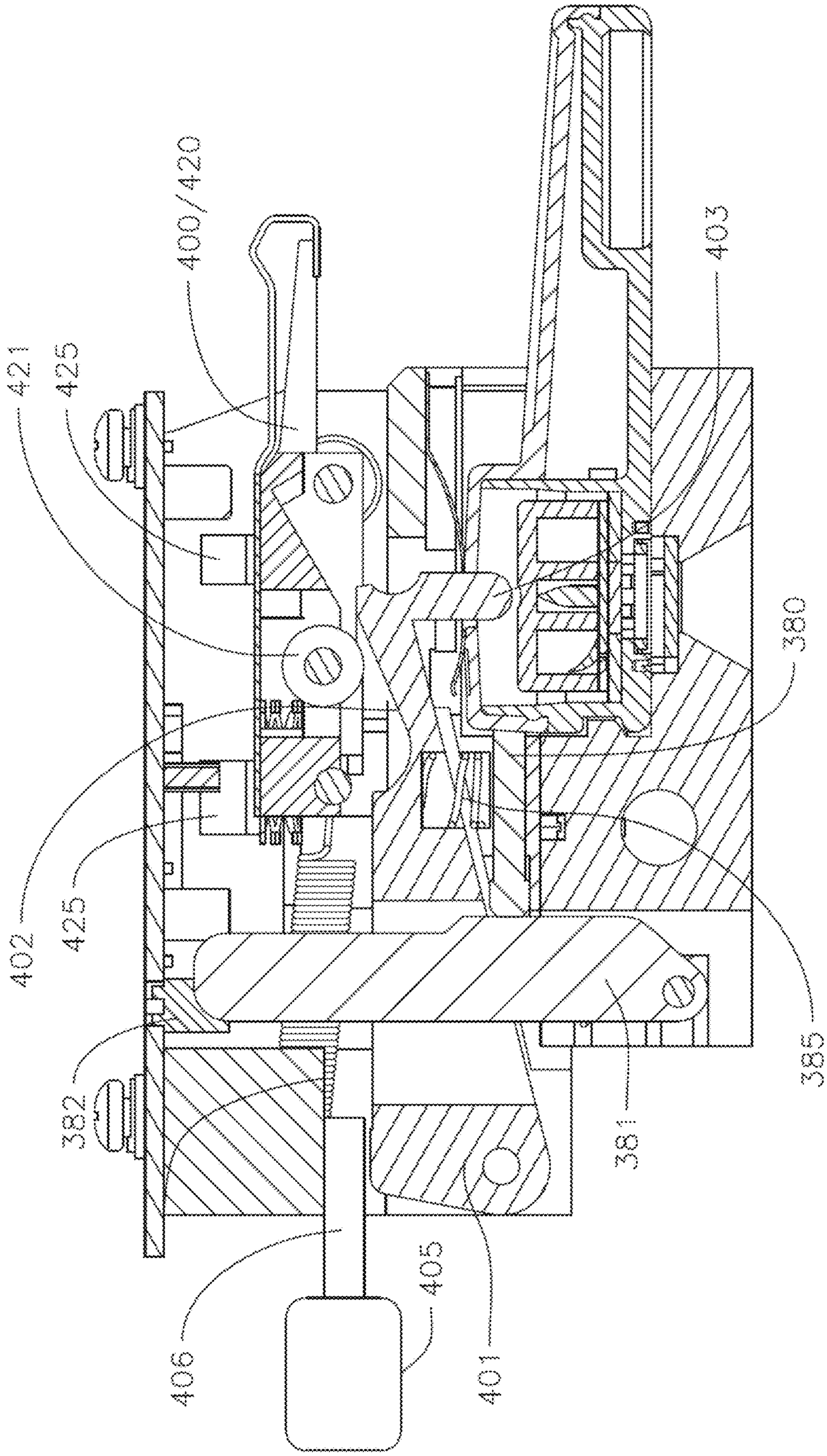


FIG. 43

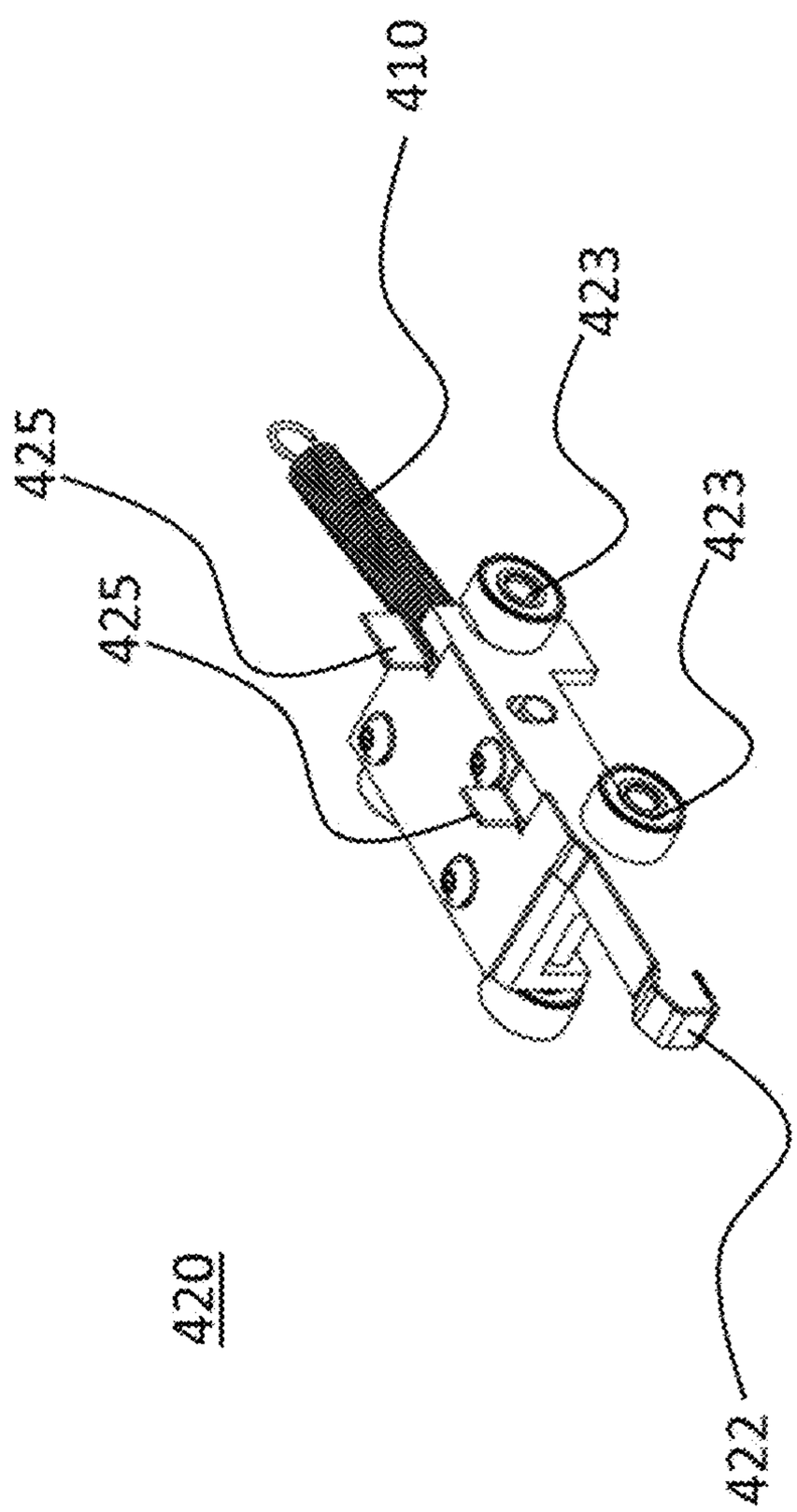


FIG. 44

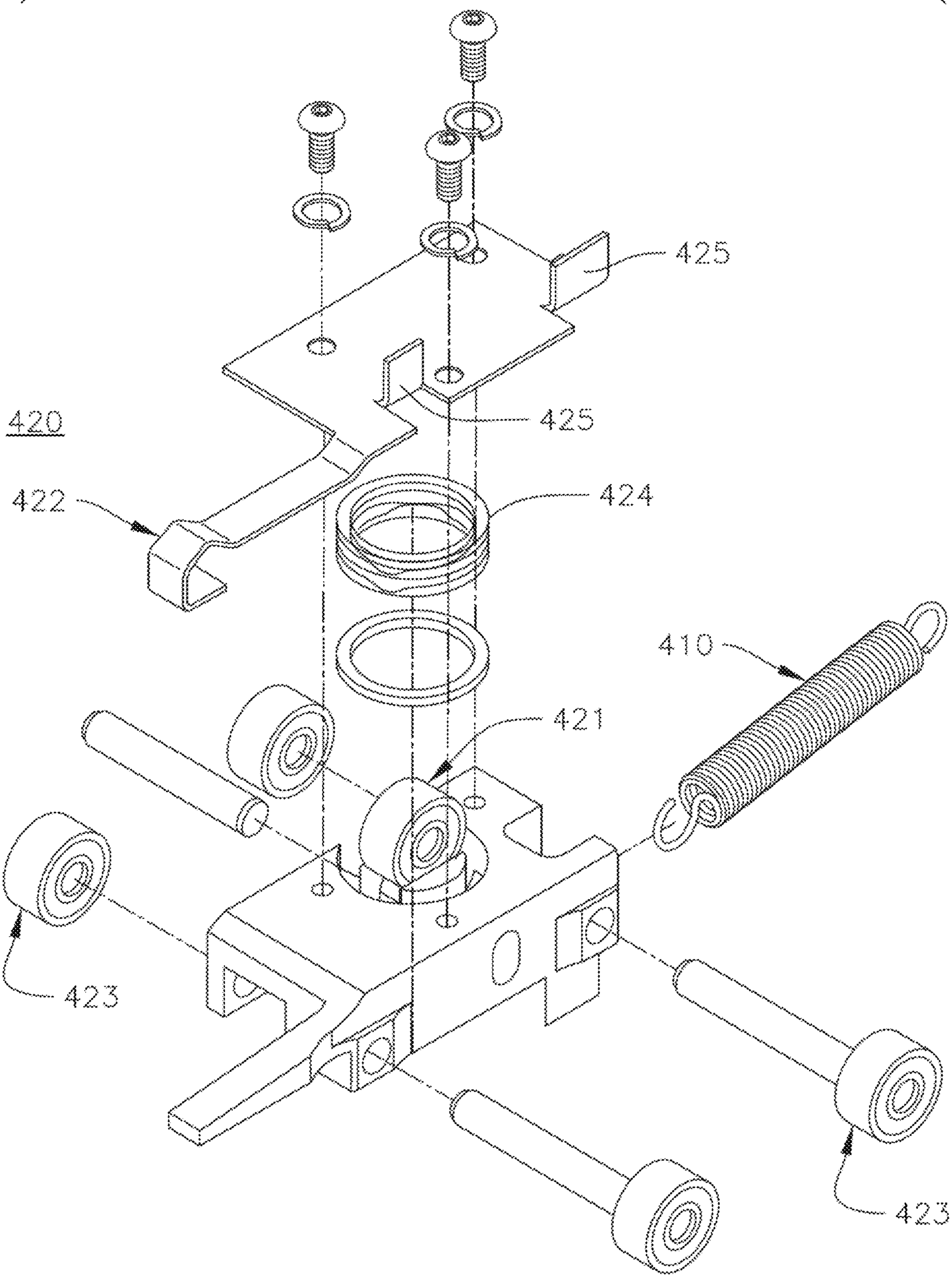


FIG. 45

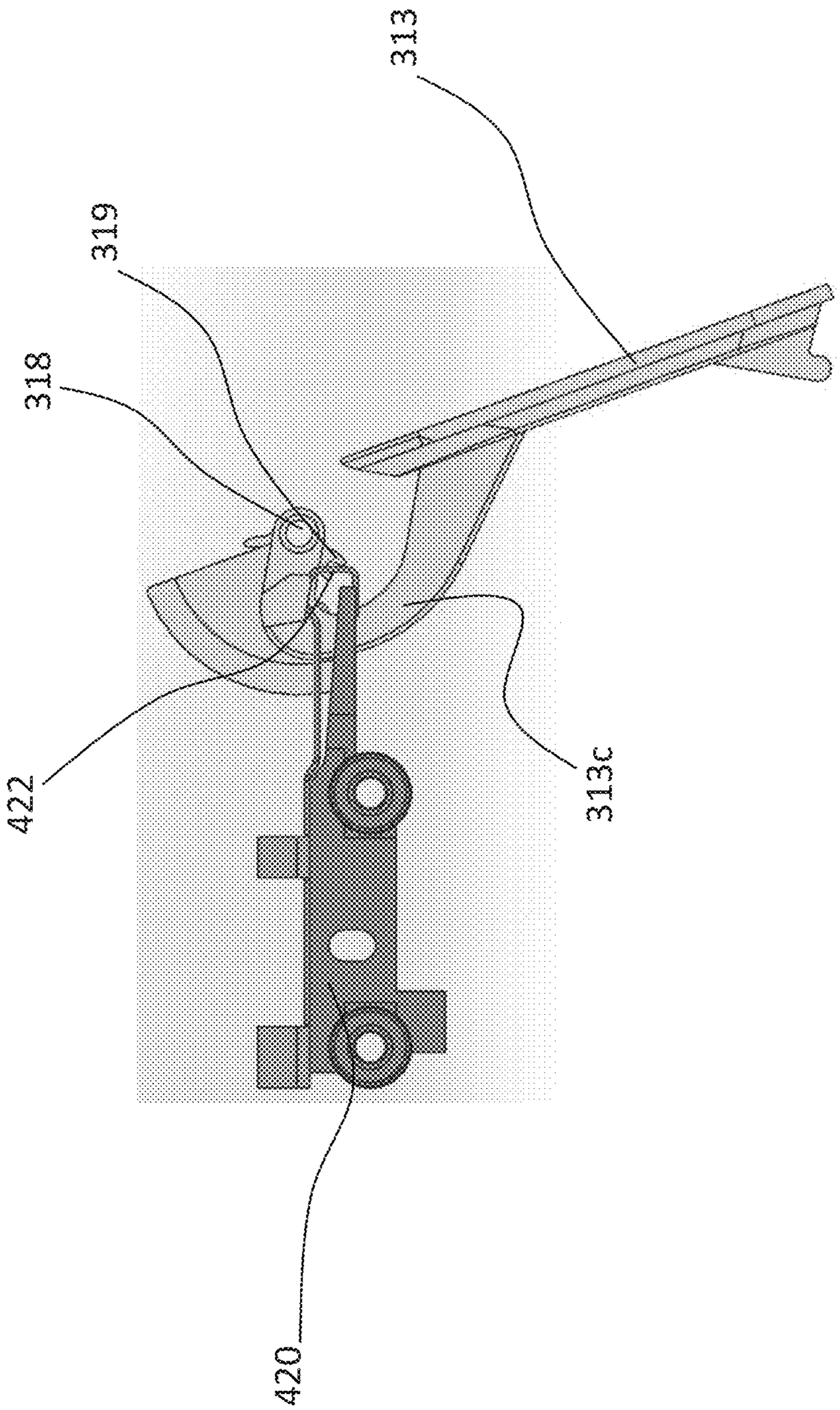
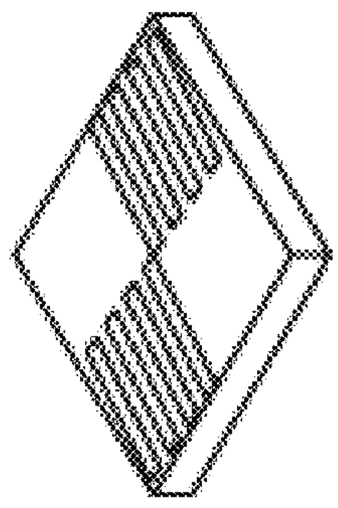


FIG. 46



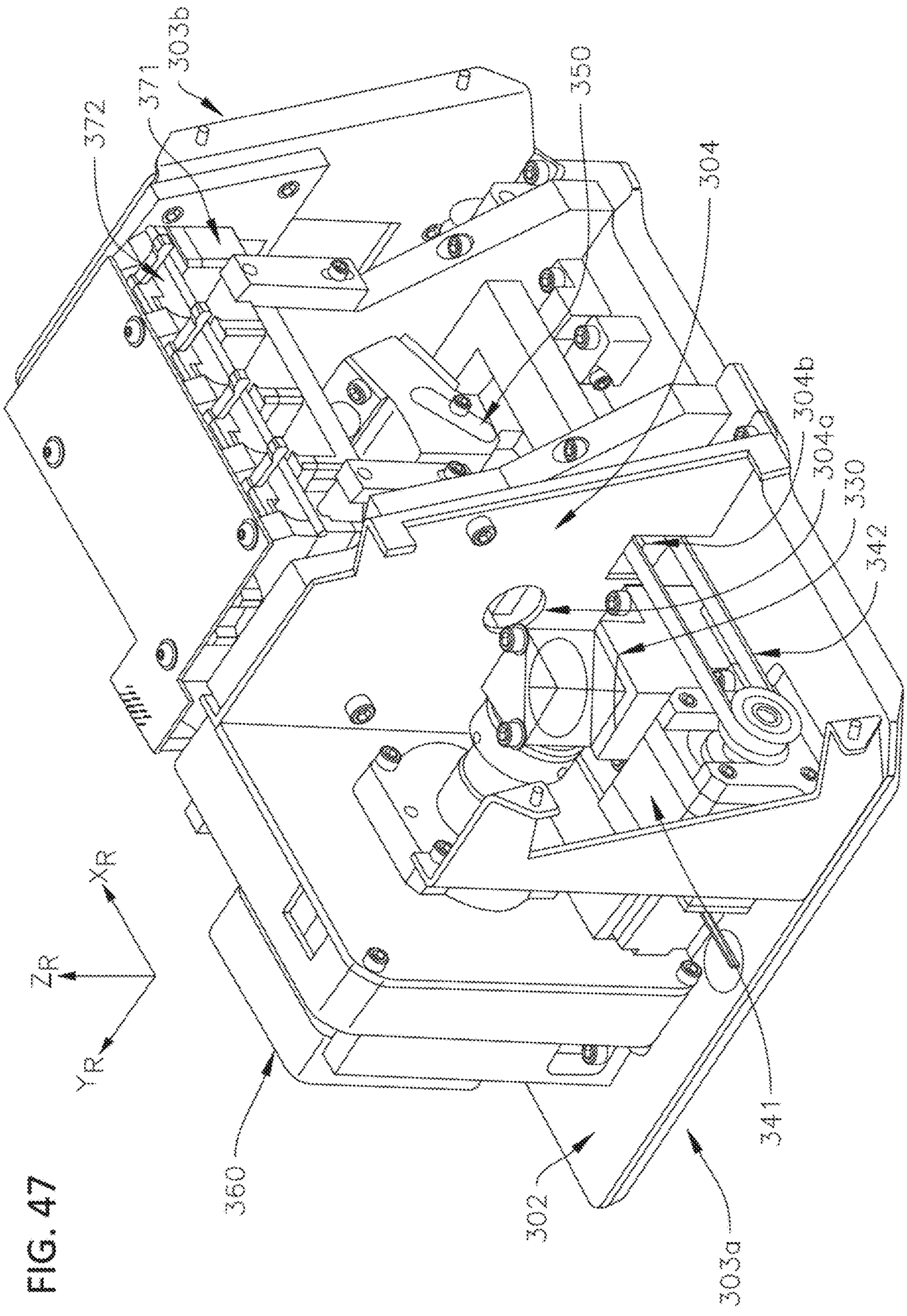


FIG. 47

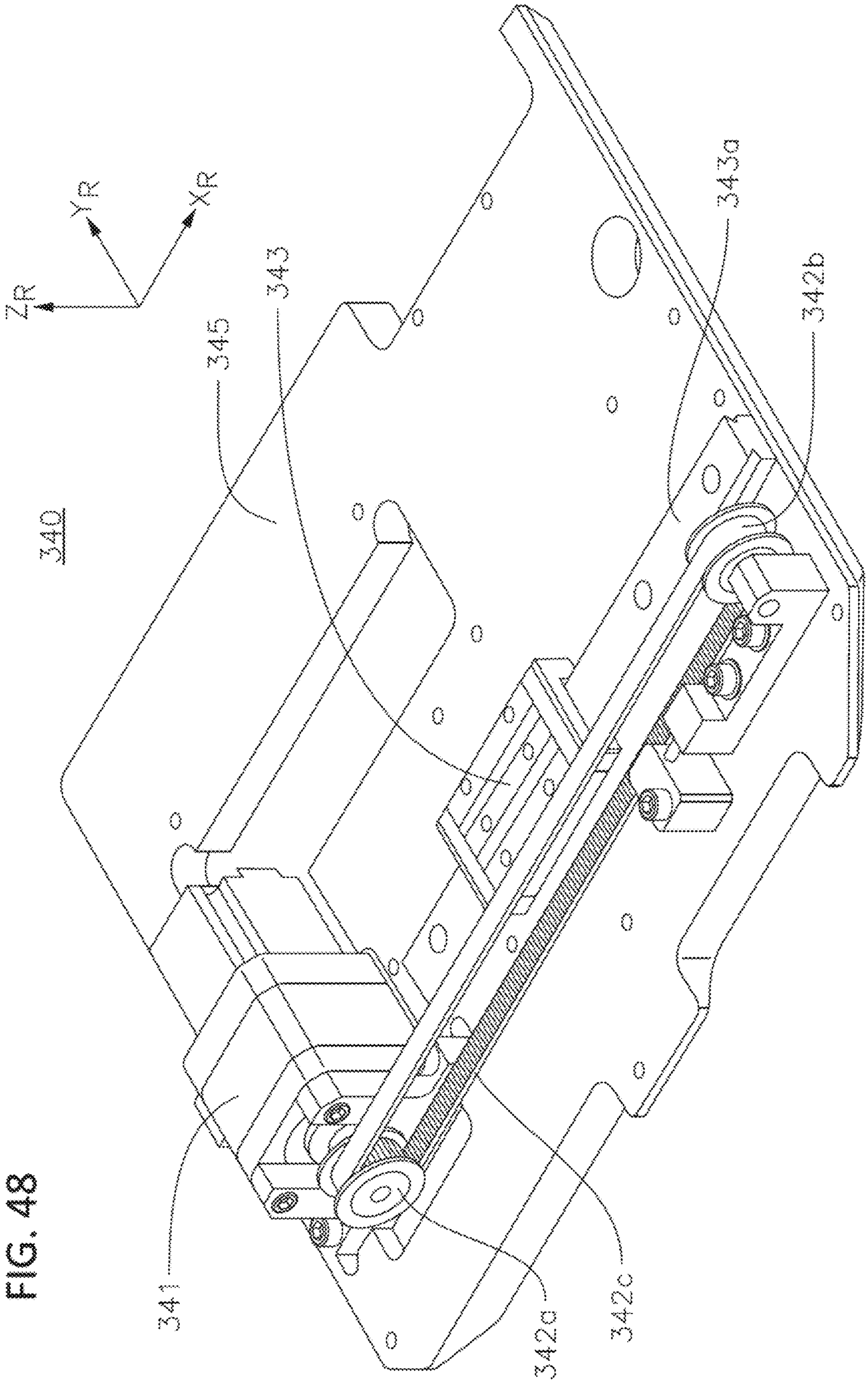


FIG. 48

FIG. 49

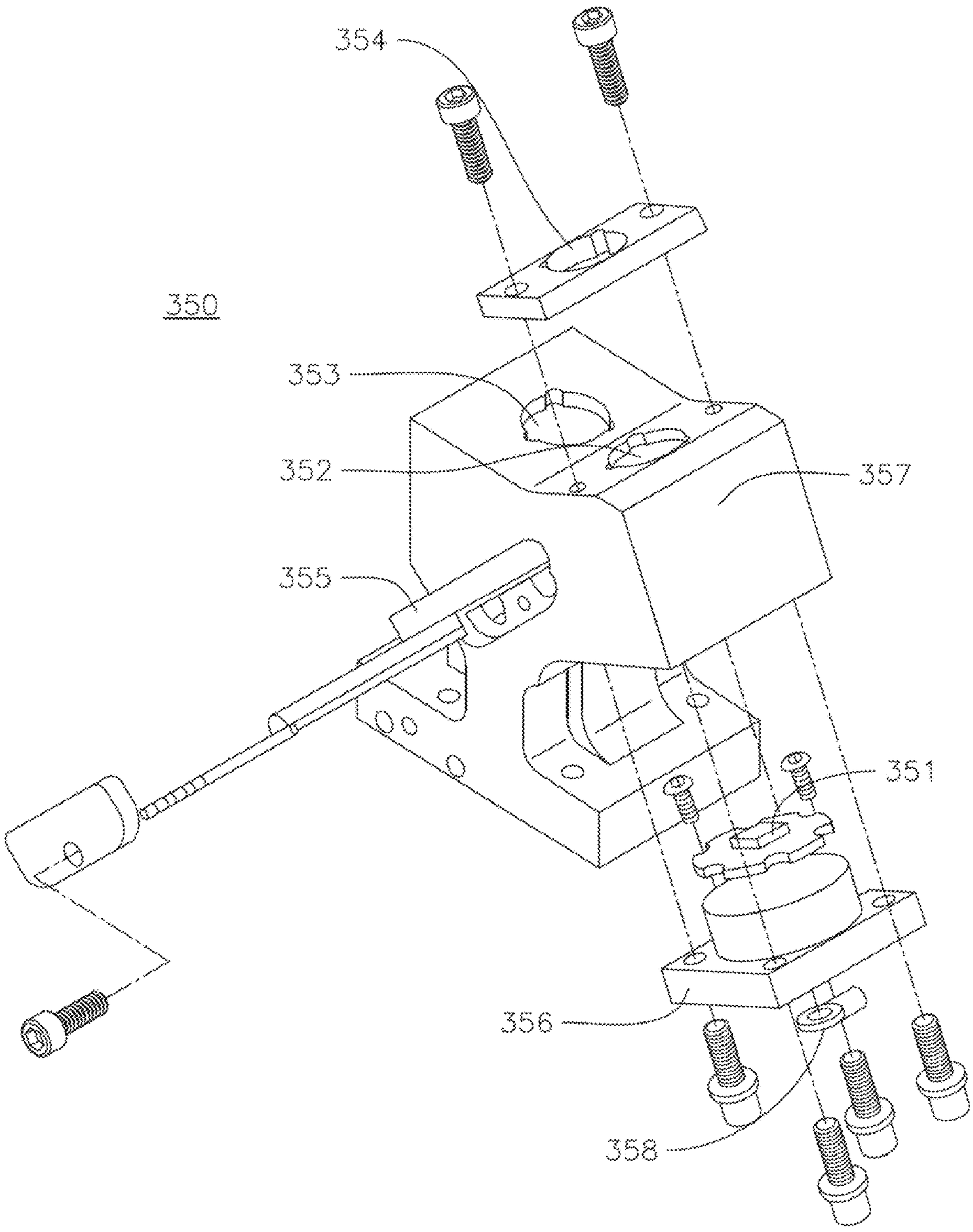


FIG. 50

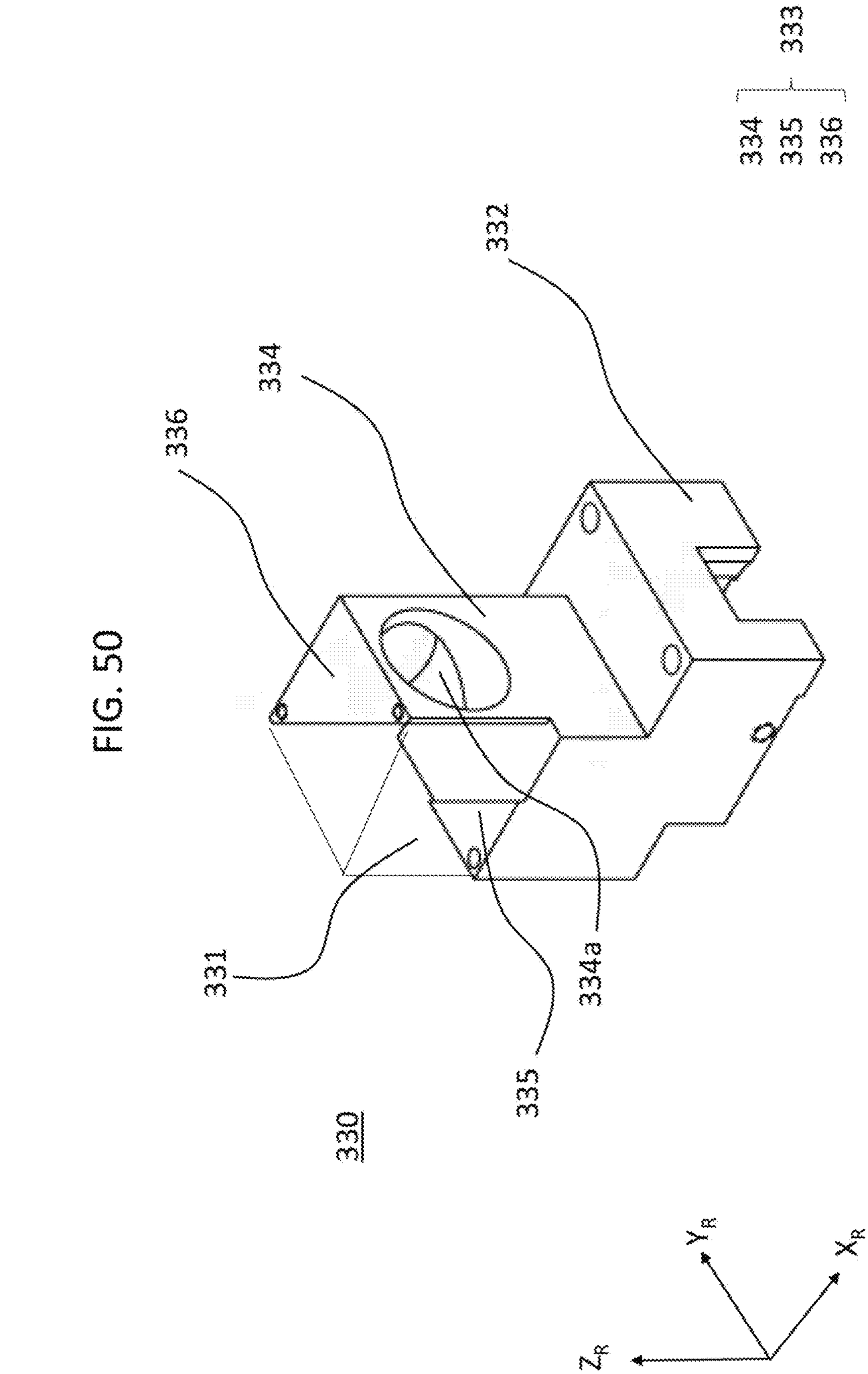
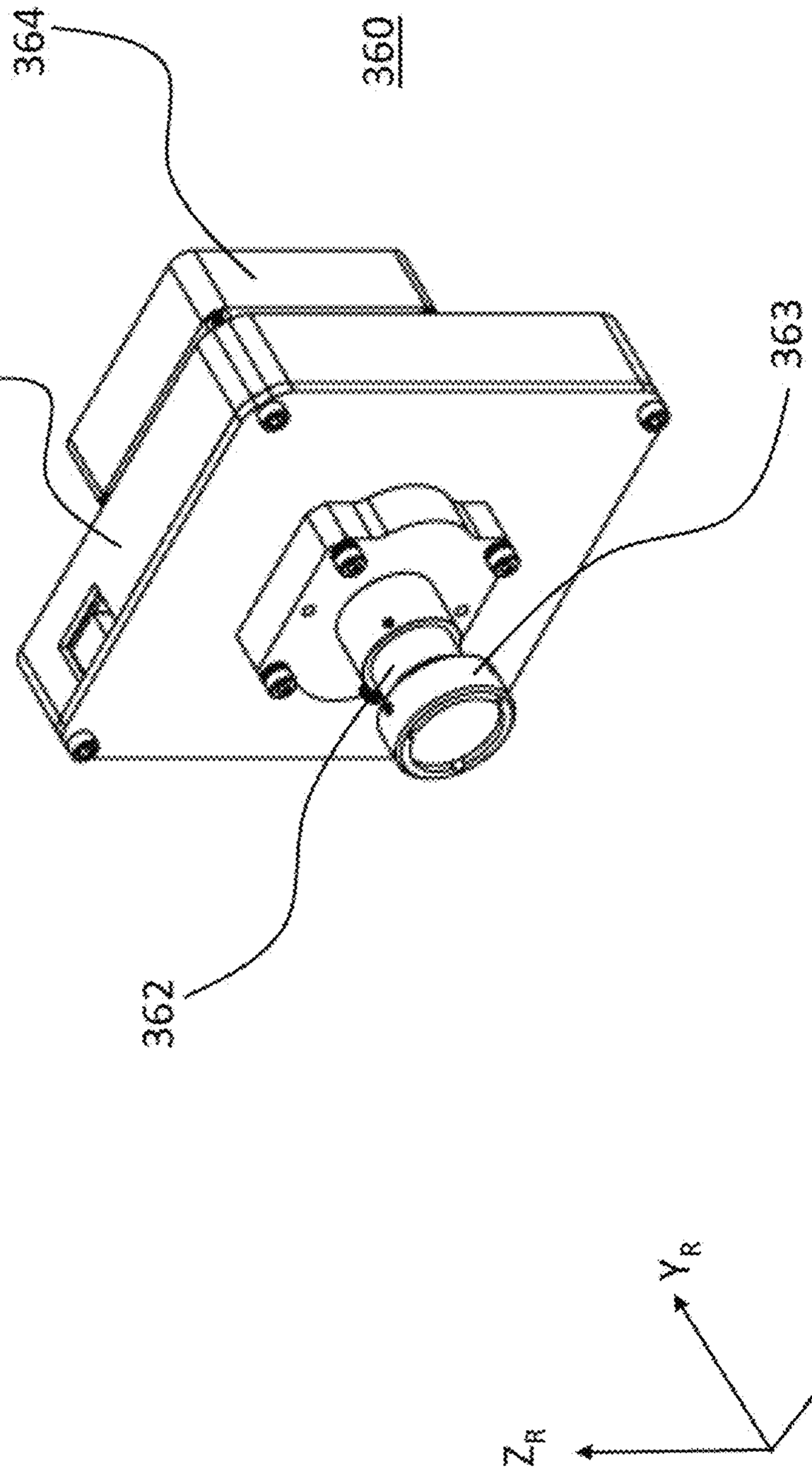


FIG. 51A



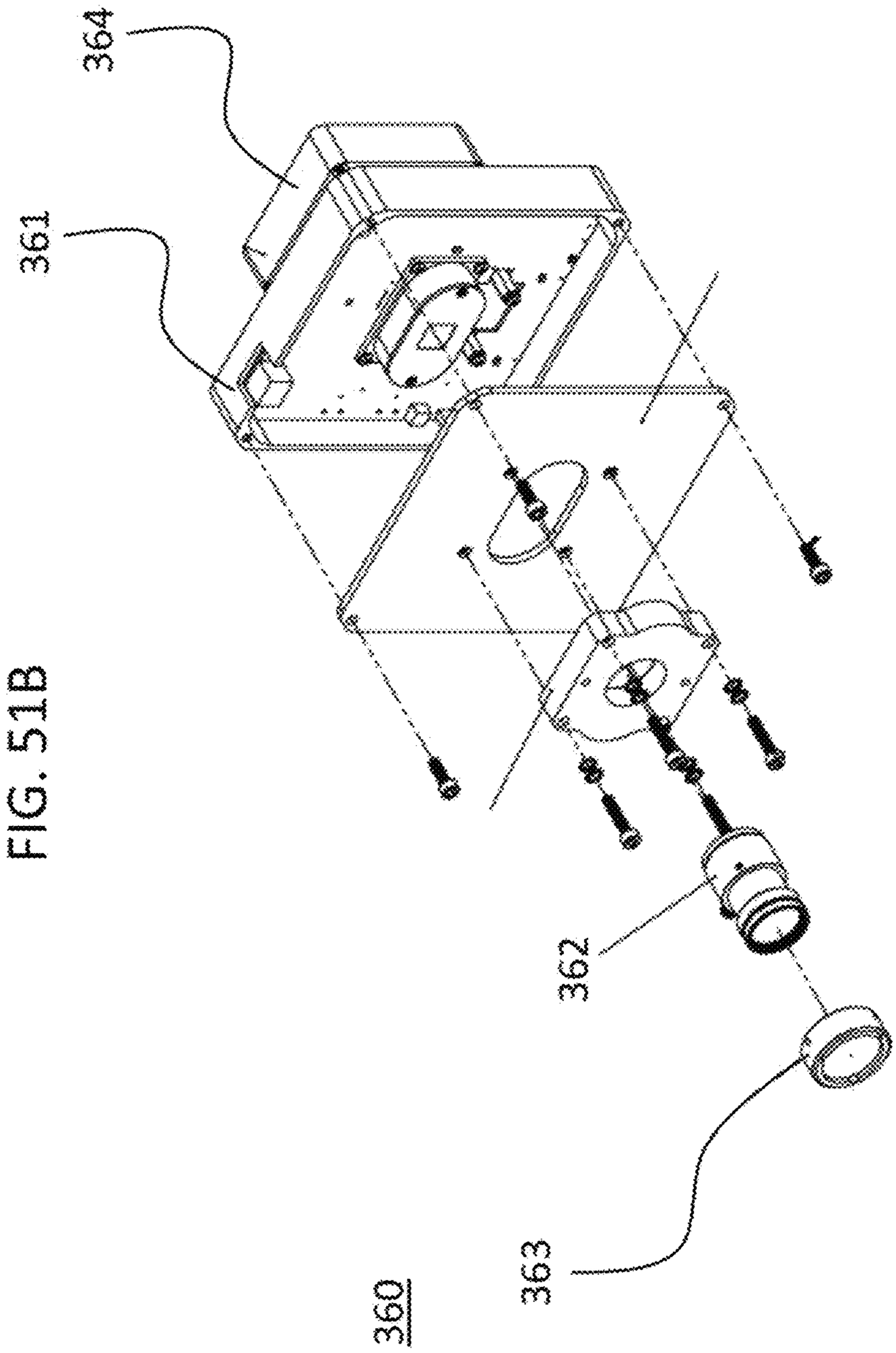


FIG. 51B

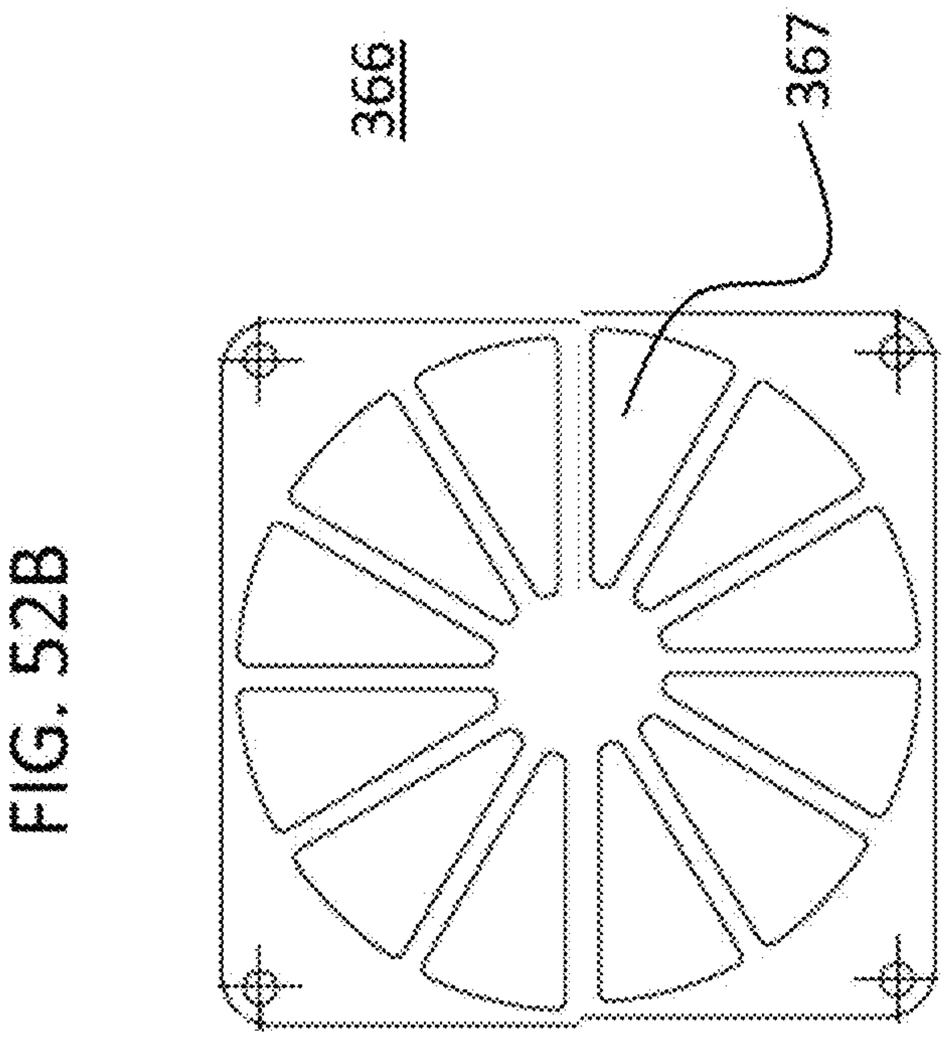


FIG. 52B

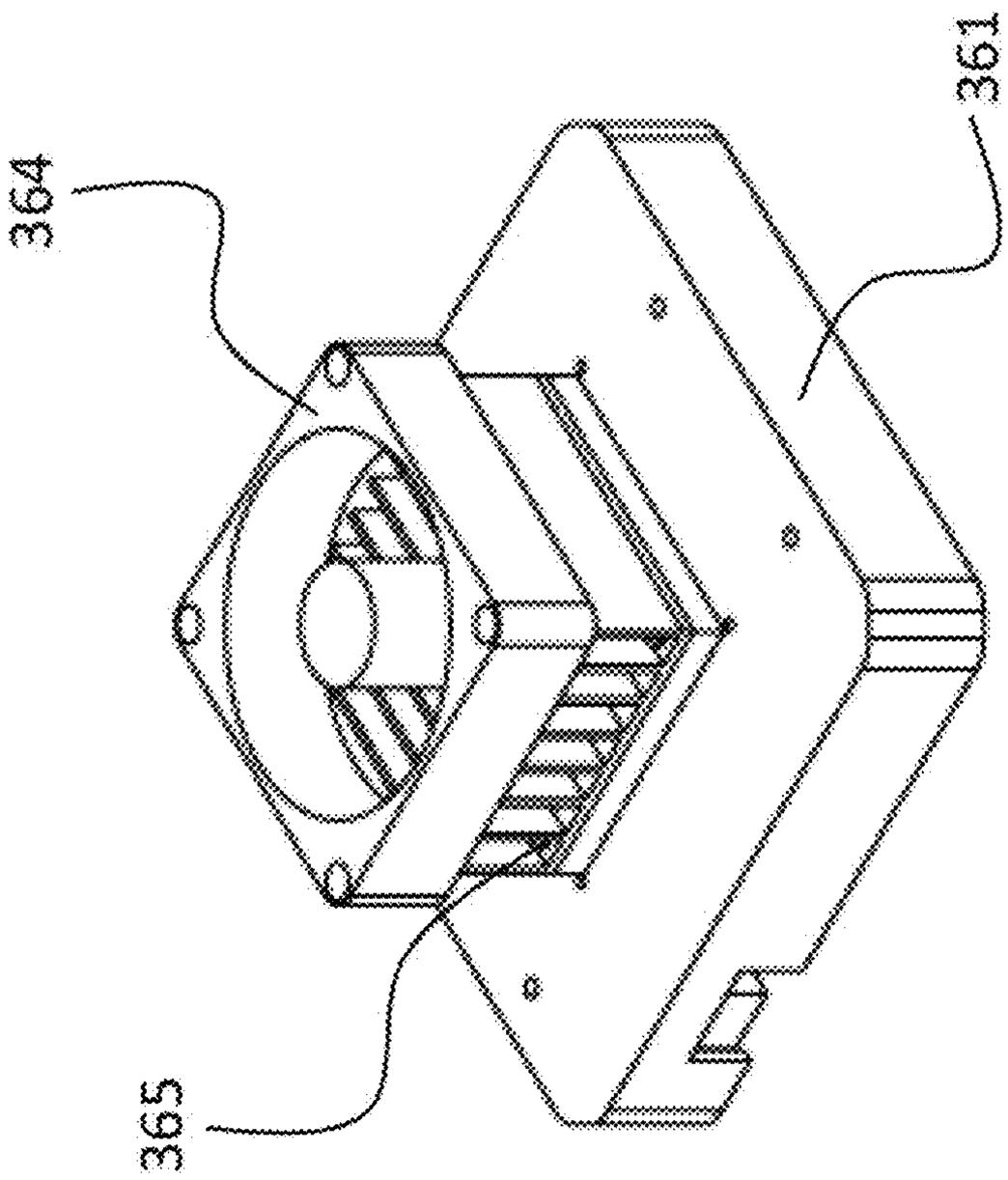


FIG. 52A

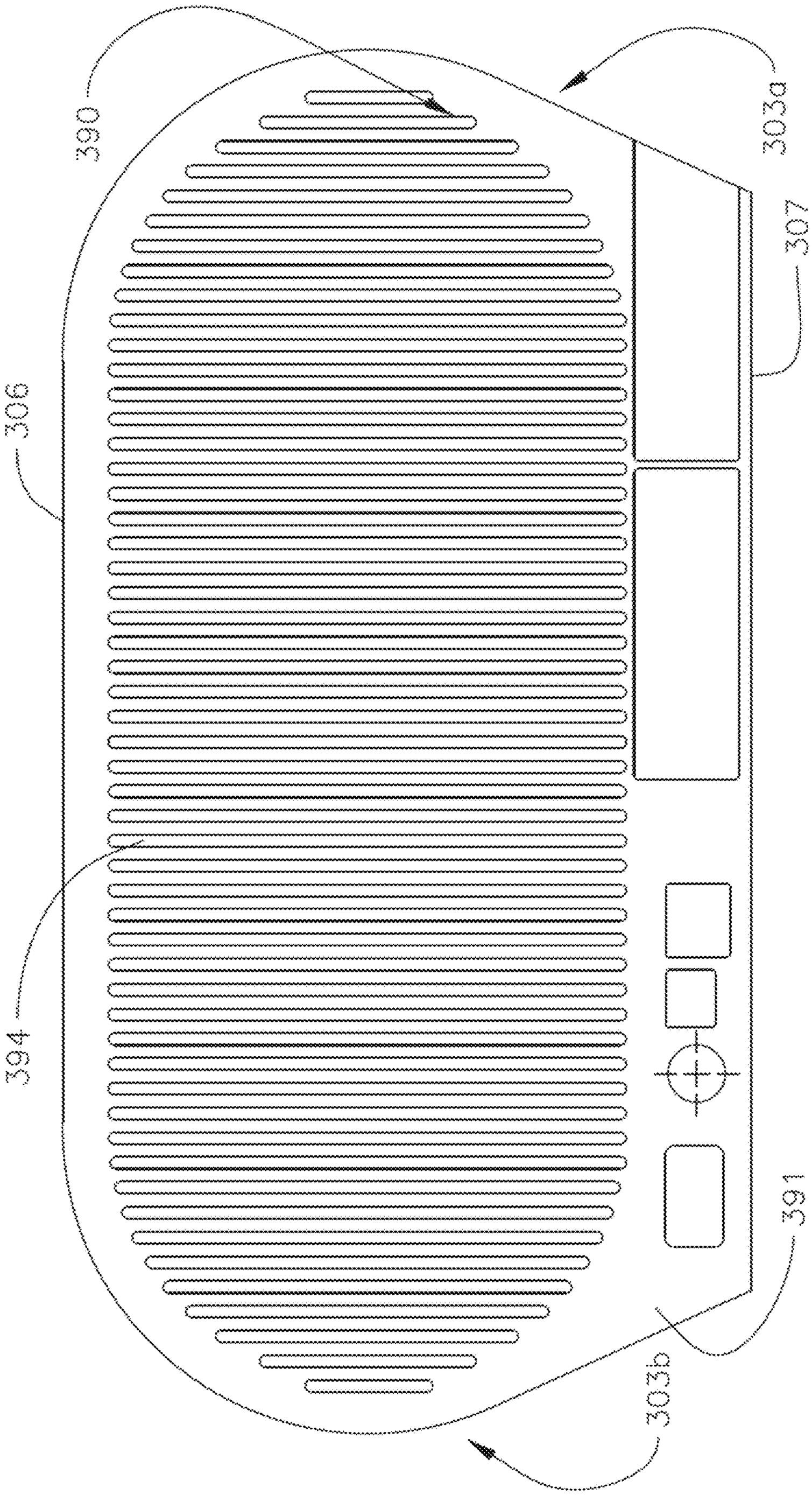


FIG. 53

FIG. 54

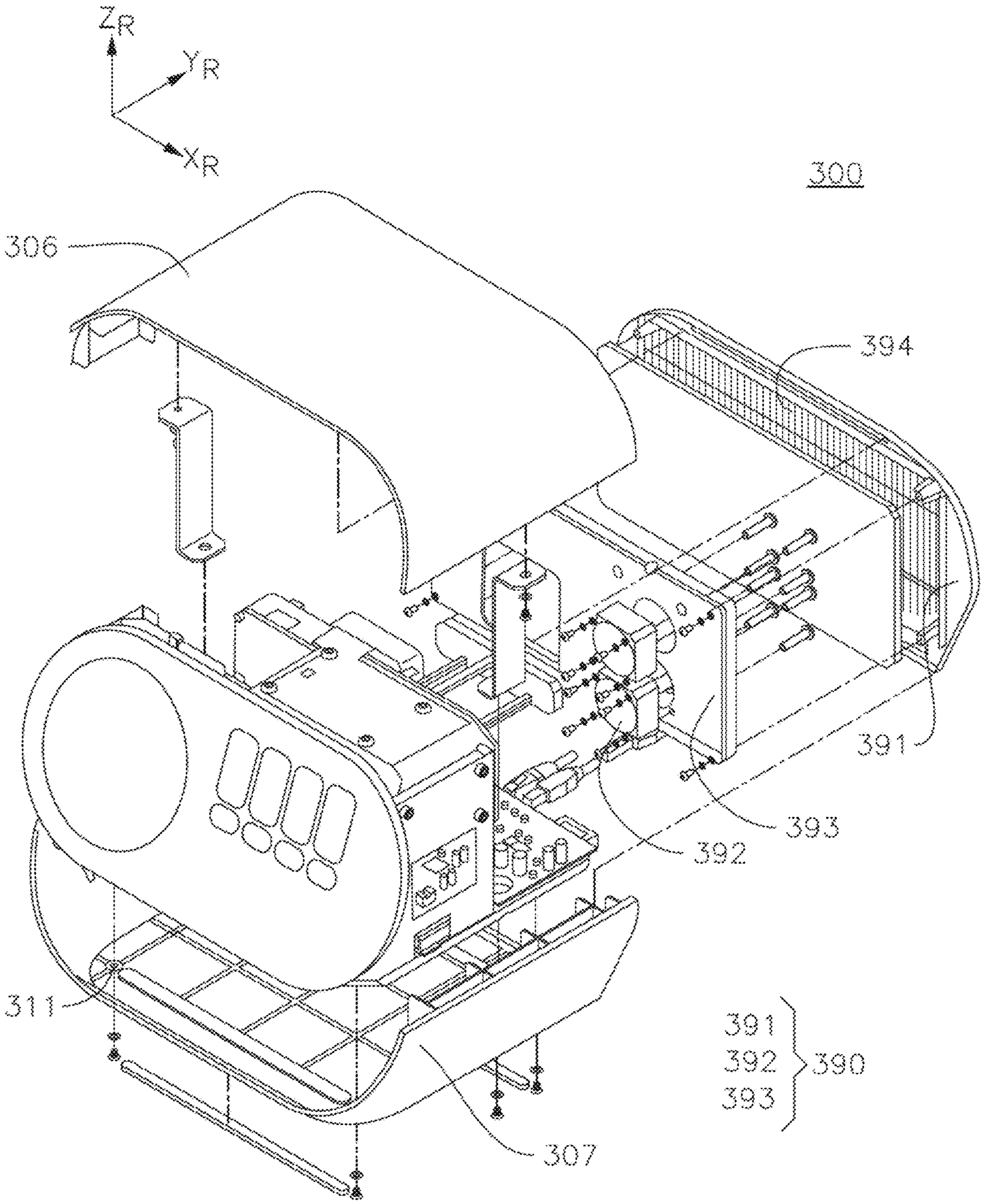


FIG. 55

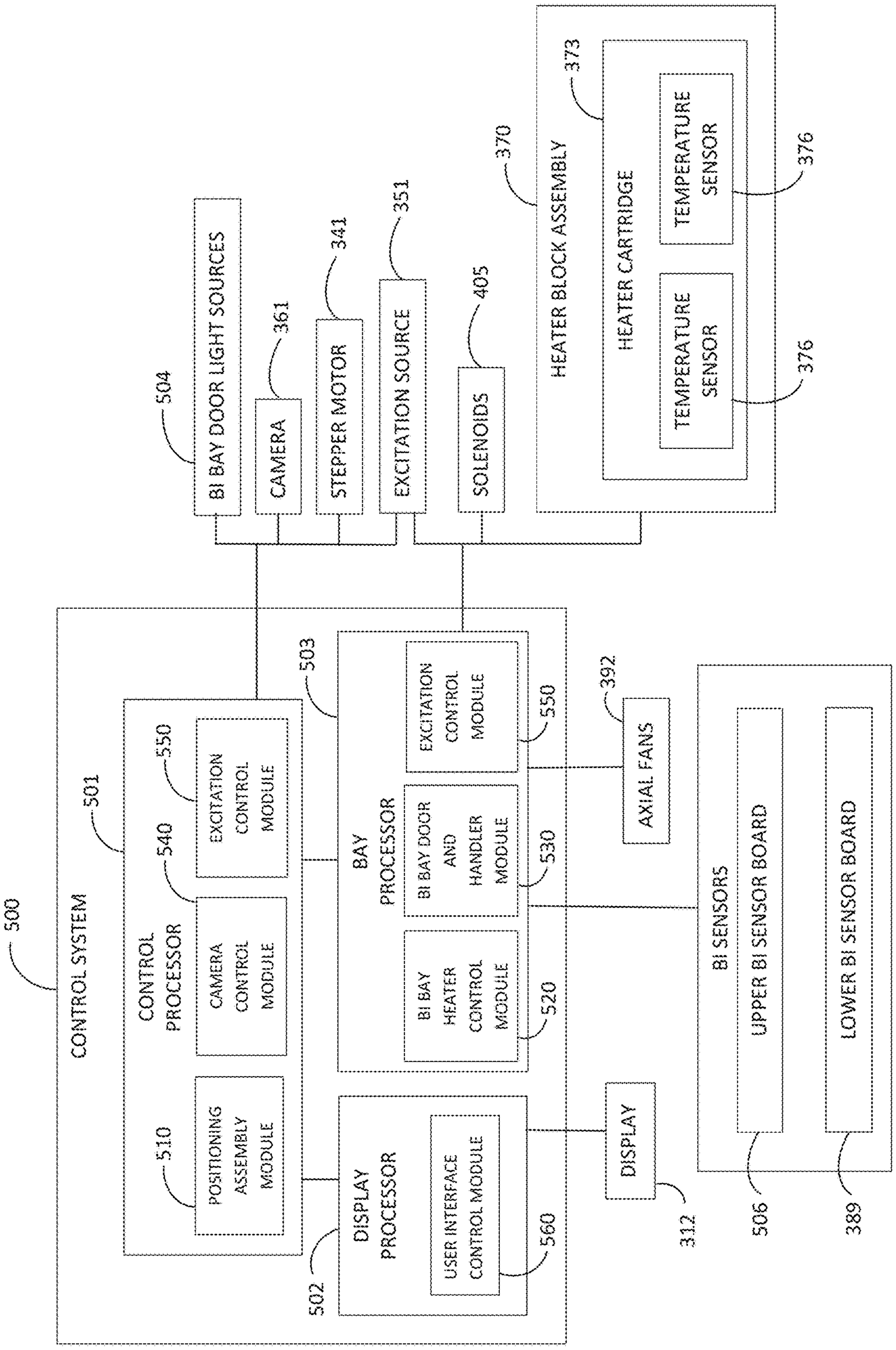

500
CONTROL SYSTEM
501
CONTROL PROCESSOR
540
CAMERA CONTROL MODULE
550
EXCITATION CONTROL MODULE
POSITIONING ASSEMBLY MODULE
510
503
BAY PROCESSOR
BI BAY DOOR AND HANDLER MODULE
530
EXCITATION CONTROL MODULE
550
BI BAY HEATER CONTROL MODULE
520
502
DISPLAY PROCESSOR
USER INTERFACE CONTROL MODULE
560
504
BI BAY DOOR LIGHT SOURCE
CAMERA
361
STEPPER MOTOR
341
EXCITATION SOURCE
351
SOLENOIDS
405
370
HEATER BLOCK ASSEMBLY
373
HEATER CARTRIDGE
TEMPERATURE SENSOR
376
TEMPERATURE SENSOR
376
392
AXIAL FANS
BI SENSORS
UPPER BI SENSOR BOARD
506
LOWER BI SENSOR BOARD
389
DISPLAY
312

FIG. 56

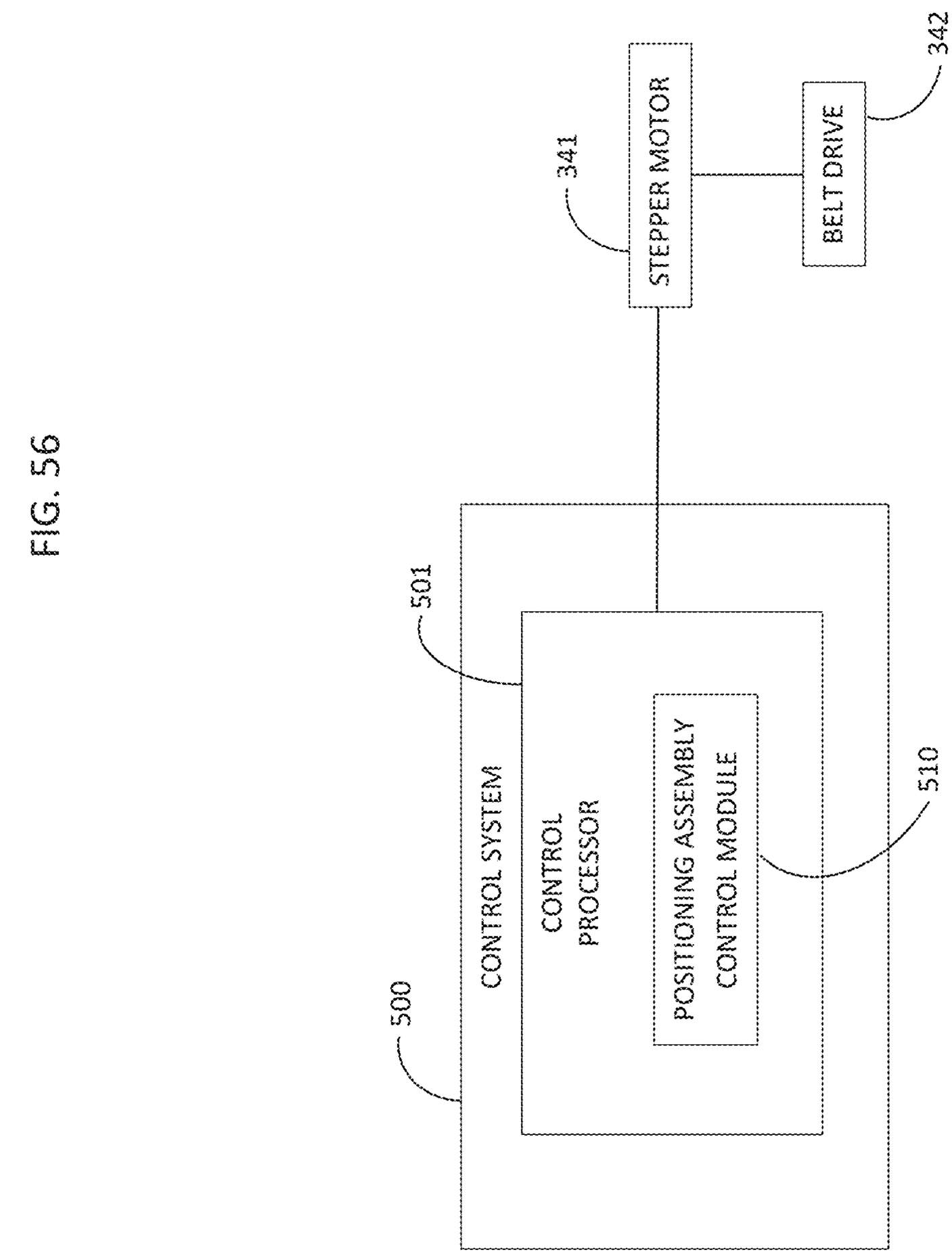

500
CONTROL SYSTEM
501
CONTROL PROCESSOR
POSITIONING ASSEMBLY CONTROL MODULE
510
STEPPER MOTOR
341
BELT DRIVE
342

FIG. 57

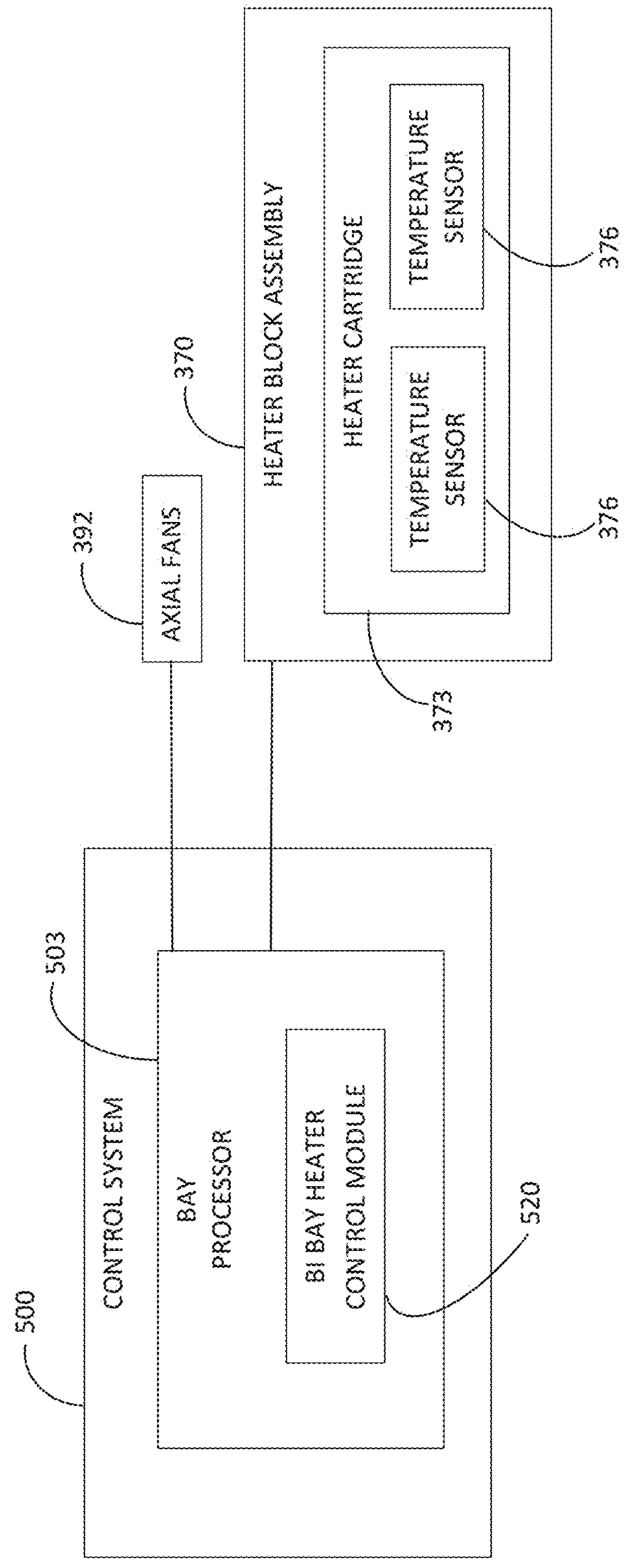

CONTROL SYSTEM
500
BAY
PROCESSOR
503
BI BAY HEATER
CONTROL MODULE
520
AXIAL FANS
392
HEATER BLOCK ASSEMBLY
370
HEATER CARTRIDGE
373
TEMPERATURE
SENSOR
376
TEMPERATURE
SENSOR
376

FIG. 58

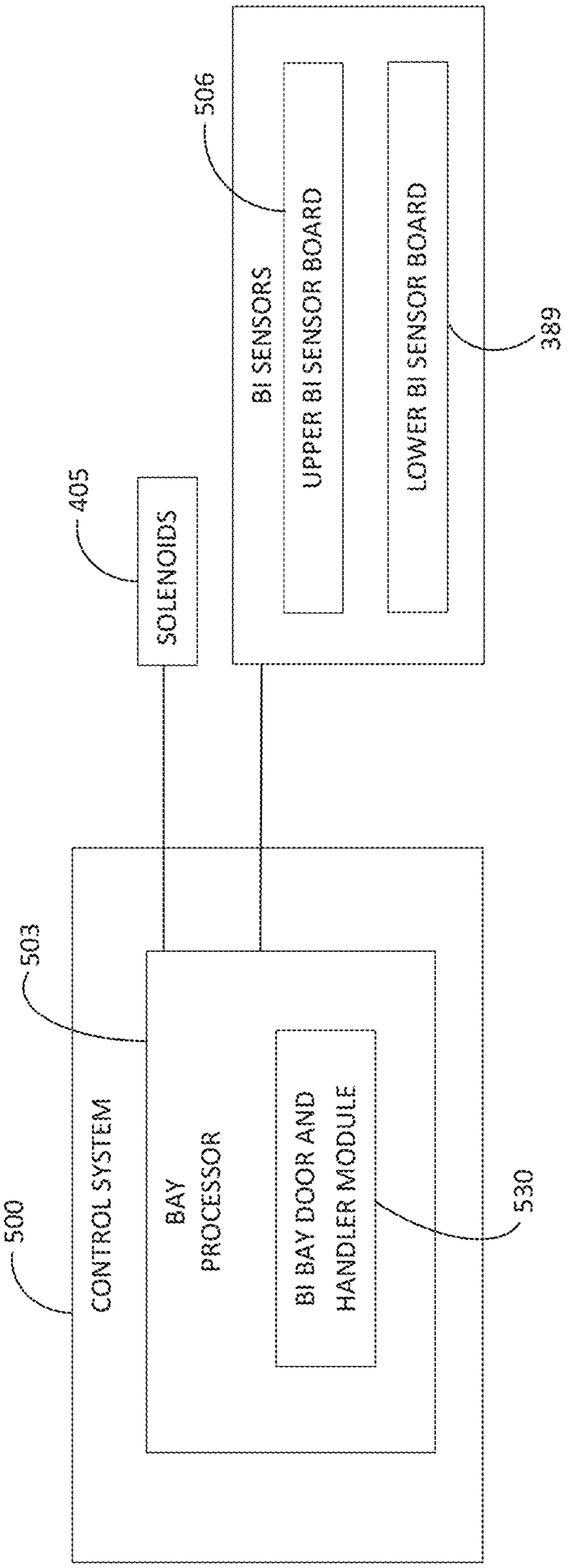

CONTROL SYSTEM
500
BAY
PROCESSOR
503
BI BAY DOOR AND
HANDLER MODULE
530
SOLENOIDS
405
BI SENSORS
506
UPPER BI SENSOR BOARD
LOWER BI SENSOR BOARD
389

FIG. 59

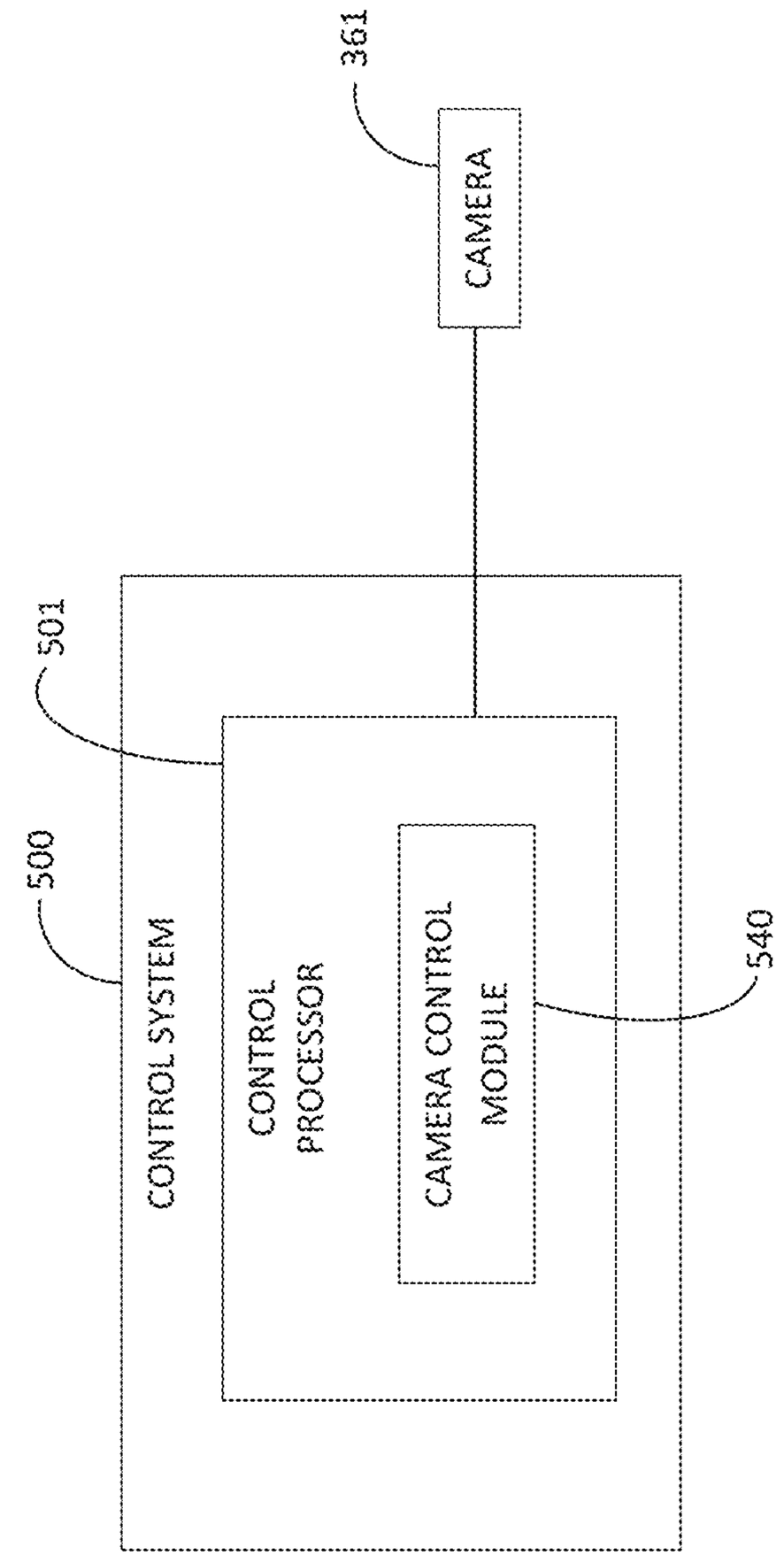

361
CAMERA
501
500
CONTROL SYSTEM
CONTROL PROCESSOR
CAMERA CONTROL MODULE
540

FIG. 60

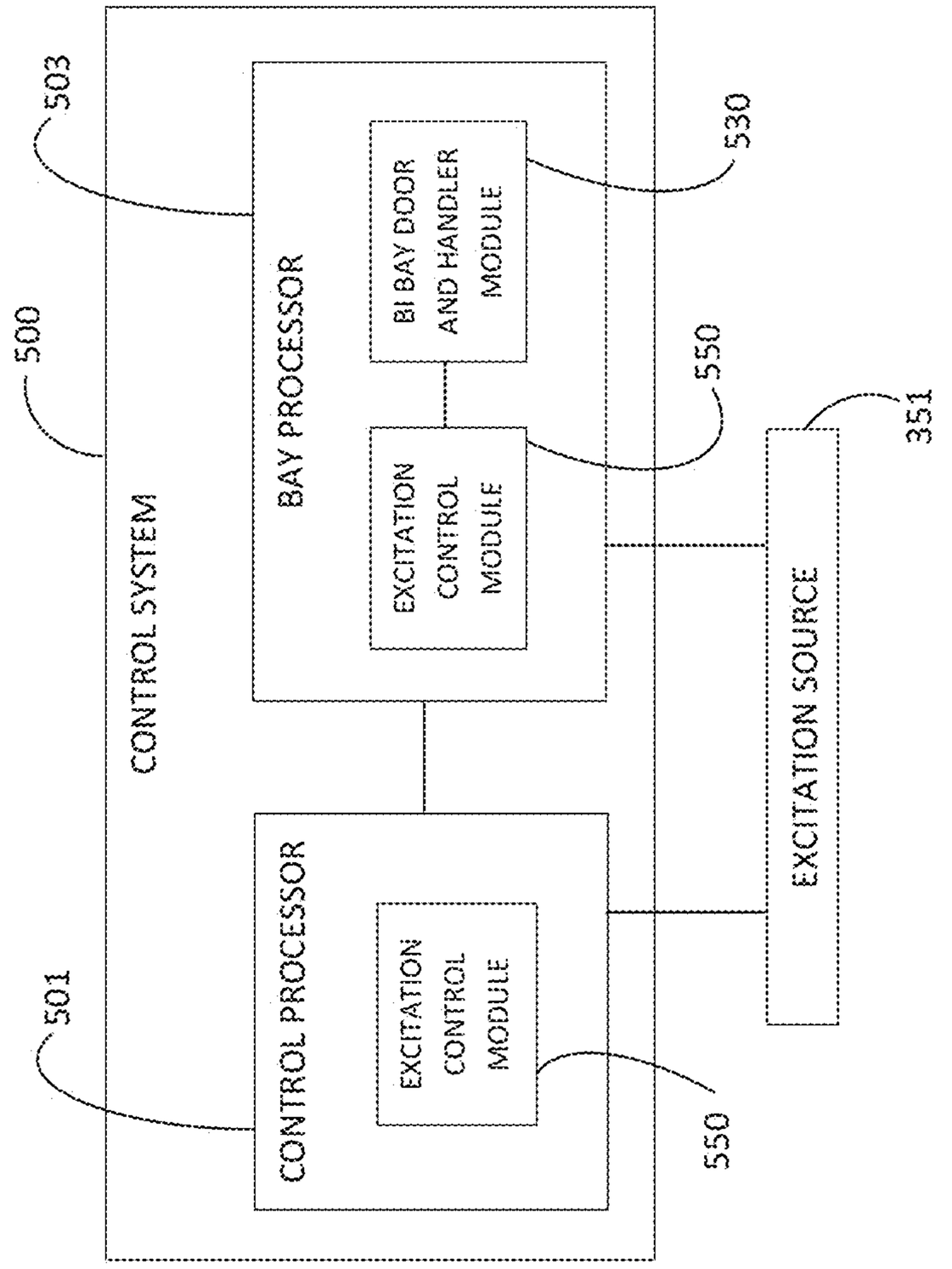
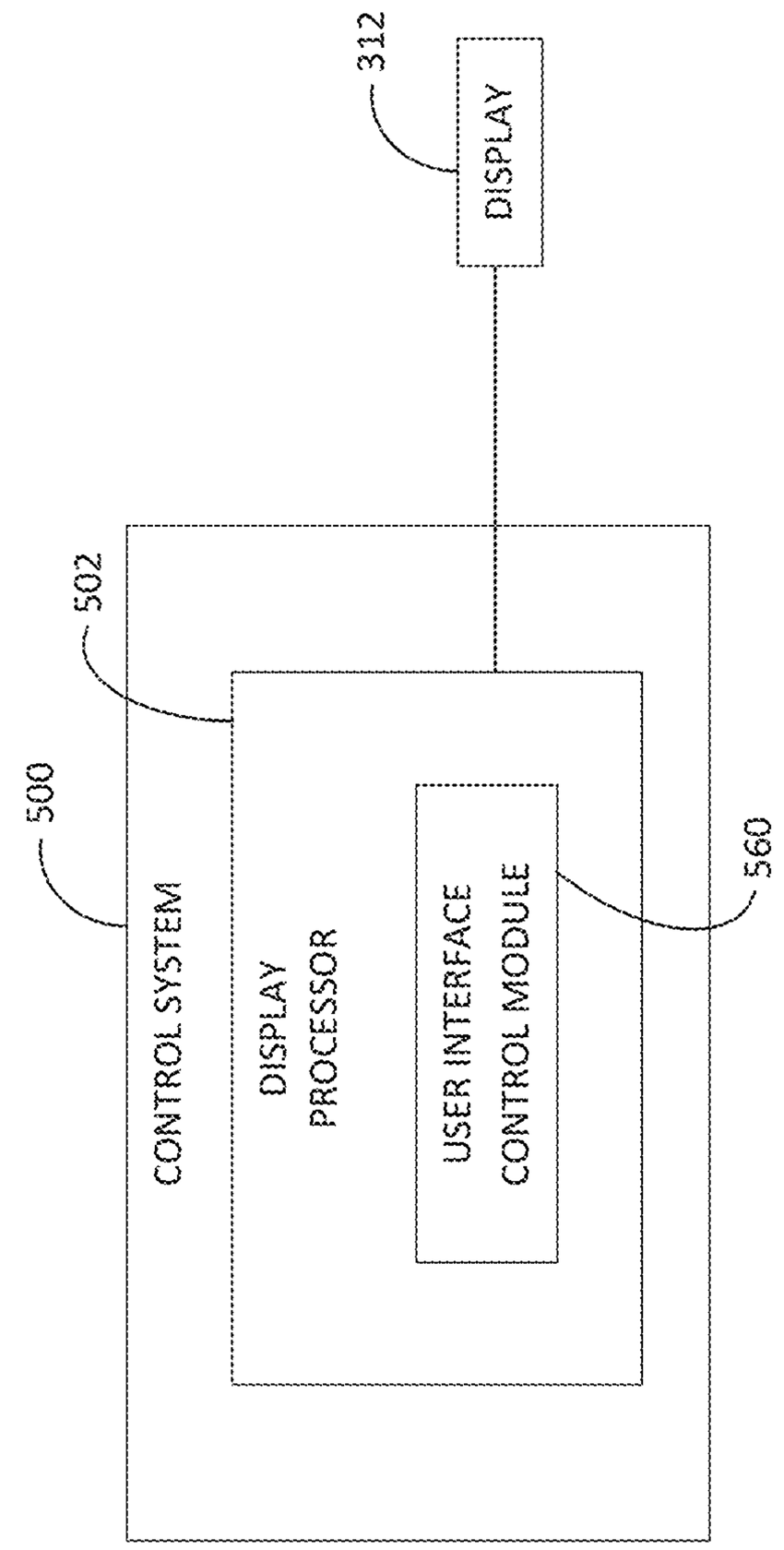

500
CONTROL SYSTEM
503
BAY PROCESSOR
BI BAY DOOR AND HANDLER MODULE
530
EXCITATION CONTROL MODULE
550
351
EXCITATION SOURCE
501
CONTROL PROCESSOR
EXCITATION CONTROL MODULE
550

FIG. 61


312
DISPLAY
502
500
CONTROL SYSTEM
DISPLAY PROCESSOR
USER INTERFACE CONTROL MODULE
560

BIOLOGICAL INDICATORS, AND SYSTEMS AND METHODS FOR DETERMINING EFFICACY OF STERILIZATION

BACKGROUND

Several industries require sterilization of certain equipment before that equipment can be reused. One of the largest, and most recognizable, industries with such a requirement is the medical industry, which requires sterilization of various equipment—ranging from surgical instruments to routine medical devices to certain implants—to ensure safety for use. In general, sterilization procedures are designed to kill all viable living organisms within a sterilization chamber. However, sterilization can be challenging, as objects can be contaminated with numerous different types of bacteria, which carry varying levels of danger and difficulty to kill. As such, it is common (and in some industries required) to test the efficacy of each sterilization run to determine if the run successfully sterilized the equipment subjected to the run.

To assess whether a sterilization run was successful (e.g., achieved adequately lethal conditions), sterilization indicators are typically subjected to the sterilization process together with the equipment being sterilized. These sterilization indicators are then analyzed to determine whether the sterilization run associated with the co-processed equipment was successful. One type of sterilization indicator is known as a chemical indicator, which responds to one or more of the critical parameters of a sterilization process and typically either changes color or has a moving front with an endpoint to provide information concerning the sterilization process. Chemical indicators, however, only provide a rough proxy for sterilization success, and therefore may be unreliable.

Another type of sterilization indicator is known as a biological indicator (or "bioindicator"). Biological indicators typically include a population of bacterial spores enclosed in the indicator, which is subjected to the same sterilization run as the equipment being sterilized. Current sterility assurance technologies that make use of biological indicators utilize assays that require at least one day for direct (and at least 20 minutes for indirect) measurements of microorganism survival within the biological indicator. Most of these assays rely on indirect measurement of microorganism survival, and do not quantify the microorganism survival. For example, indirect measurements test for a global change in a specified metric, such as fluorescence, which is then used to determine whether sterility was likely effective. However, the accuracy of such indirect measurements is susceptible to exogenous factors unrelated to the biological changes of interest, which renders these indirect methods less reliable. Additionally, current sterility assurance technologies often rely on these nonquantitative measurements of microorganism survival, and simply return a positive result (indicating microorganism survival and therefore sterilization failure) or a negative result (indicating no detected microorganism survival and therefore sterilization success). And due to the nature of these conventional assays, the positive or negative result can only be returned after the 24 hour (for direct measurement) or 20 minute (for indirect measurement) period.

SUMMARY OF THE INVENTION

According to embodiments of the present disclosure, devices, systems and methods for determining the efficacy of a sterilization process (or "run") enable sterility assurance

results to be returned within a fraction of the time currently needed using conventional tools and methods. Aspects of embodiments of the present disclosure are directed to a biological indicator, a process challenge device, and a biological indicator reader having improved accuracy for determining the efficacy of a sterilization process (or "run"). Aspects of embodiments of the present disclosure provide for sterility testing of multiple biological indicators in the biological indicator reader concurrently, allowing for relatively quick sterility assurance with the same equipment. Aspects of embodiments of the present disclosure also provide for a biological indicator and biological indicator reader that provides a direct reading of the presence of live spore(s) in the biological indicator following sterilization.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are included to provide a further understanding of example embodiments of the present disclosure, and are incorporated in, and form a part of this specification. The drawings illustrate exemplary embodiments of the present disclosure and, together with the description, serve to explain principles of the inventive concept(s) of the present disclosure. In the drawings, like reference numerals refer to like elements throughout, unless otherwise specified. In the drawings:

FIG. **1** is a perspective view of a biological indicator (BI) according to embodiments of the present disclosure;

FIG. **2** is a side elevational view of the biological indicator (BI) of FIG. **1**;

FIG. **3** is a top plan view of a first shell of the biological indicator (BI) of FIG. **1**;

FIG. **4** is a cross-sectional view of the first shell of FIG. **3** taken along the line IV-IV of FIG. **3**;

FIG. **5** is a top plan view of a second shell of the biological indicator (BI) of FIG. **1**;

FIG. **6** is a cross-sectional view of the second shell of FIG. **5** taken along the line VI-VI of FIG. **5**;

FIG. **7** is a bottom plan view of the second shell of FIG. **5**;

FIG. **8** is a perspective view of a germinant releaser support according to embodiments of the present disclosure;

FIG. **9** is a top plan view of the germinant releaser support of FIG. **8**;

FIG. **10** is a cross-sectional view of the germinant releaser support of FIG. **9** taken along the line X-X of FIG. **9**;

FIG. **11** is a bottom plan view of the germinant releaser support of FIG. **8**;

FIG. **12** is an exploded perspective view of a biological indicator (BI) according to embodiments of the present disclosure;

FIG. **13** is an exploded perspective view of a biological indicator (BI) according to embodiments of the present disclosure;

FIG. **14** is a cross-sectional view of a second shell of the biological indicator (BI) of FIG. **13**;

FIG. **15** is a perspective view of a germinant container of the biological indicator (BI) of FIG. **13**;

FIG. **16**A is a top plan view of the germinant container of FIG. **15**;

FIG. **16**B is a bottom plan view of the germinant container of FIG. **15**;

FIG. **17** is a perspective view of a germinant releaser of the biological indicator (BI) of FIG. **13**;

FIG. **18** is a top plan view of the germinant releaser of FIG. **17**;

FIG. **19** is a bottom plan view of a process challenge device according to embodiments of the present disclosure;

FIG. **20** is a side elevational view of the process challenge device of FIG. **19**;

FIG. **21** is a perspective view of a tray of the process challenge device of FIG. **19**;

FIG. **22** is a top elevational view of a steam sterilization integrator according to embodiments of the present disclosure;

FIG. **23** is a perspective view of a bottom of the steam sterilization integrator of FIG. **22**;

FIG. **24** is an exploded perspective view of the process challenge device of FIG. **19**;

FIG. **25** is a perspective view of a tray of a process challenge device according to embodiments of the present disclosure;

FIG. **26** is a side elevational view of the tray of the process challenge device of FIG. **25**;

FIG. **27** is a cross-sectional view of the tray of FIG. **26** taken along the line XXVII-XXVII of FIG. **26**;

FIG. **28** is an exploded perspective view of the process challenge device of FIG. **25** and a biological indicator (BI) according to embodiments of the present disclosure;

FIG. **29** is a perspective view of a biological indicator (BI) reader according to embodiments of the present disclosure;

FIG. **30** is a front elevational view of a front surface of a front panel of the biological indicator (BI) reader of FIG. **29**;

FIG. **31** is a perspective view of a back surface of the front panel of FIG. **30**;

FIG. **32** is an exploded perspective view of a front panel assembly of the biological indicator (BI) reader of FIG. **29**;

FIG. **33** is a perspective view of an access door of the front panel assembly of the biological indicator (BI) reader of FIG. **29**;

FIG. **34** is a side view of an access door in an open configuration attached to the front panel of the biological indicator (BI) reader of FIG. **29**;

FIG. **35** is a perspective view of a heater block assembly of the biological indicator (BI) reader of FIG. **29**;

FIG. **36** is an exploded perspective view of the heater block assembly of FIG. **35**;

FIG. **37** is a top view of a biological indicator bay of a first plate of the heater block assembly of FIG. **35** prior to insertion of a biological indicator (BI) therein according to embodiments of the present disclosure;

FIG. **38** is a top view of the biological indicator (BI) bay of the first plate of the heater block assembly of FIG. **35** during insertion of the biological indicator (BI) therein according to embodiments of the present disclosure;

FIG. **39** is a top view of the biological indicator (BI) bay of the first plate of the heater block assembly of FIG. **35** after insertion of the biological indicator (BI) therein according to embodiments of the present disclosure;

FIG. **40** is a top perspective view of a second plate of the heater block assembly of FIG. **35**;

FIG. **41** is a bottom perspective view of the second plate of FIG. **40**;

FIG. **42** is a side elevational view of a biological indicator (BI) bay of the heater block assembly of FIG. **35** after insertion of a biological indicator (BI) therein and during operation of the biological indicator (BI) reader;

FIG. **43** is a perspective view of a shuttle of the heater block assembly of FIG. **35**;

FIG. **44** is an exploded perspective view of the shuttle of FIG. **43**;

FIG. **45** is a side view of a shuttle having a door interlock spring and an access door of the biological indicator (BI) reader according to embodiments of the present disclosure;

FIG. **46** is a bottom perspective view of a self-calibration target according to embodiments of the present disclosure;

FIG. **47** is a perspective view of a heater block assembly, a positioning assembly, a mirror mount, and a camera assembly according to embodiments of the present disclosure;

FIG. **48** is a perspective view of the positioning assembly of FIG. **47**;

FIG. **49** is an exploded perspective view of a scan head assembly of the positioning assembly of FIG. **47**;

FIG. **50** is a perspective view of the mirror mount of FIG. **47**;

FIG. **51**A is a perspective view of the camera assembly of FIG. **47**;

FIG. **51**B is an exploded perspective view of the camera assembly of FIG. **51**A;

FIG. **52**A is a back perspective view of the camera assembly of FIG. **47**;

FIG. **52**B is a front elevational view of a fan guard of the camera assembly of FIG. **47**;

FIG. **53** is a back elevational view of the biological indicator (BI) reader of FIG. **29**;

FIG. **54** is an exploded perspective view of the biological indicator (BI) reader of FIG. **29**;

FIG. **55** is a schematic diagram of a control system according to embodiments of the present disclosure;

FIG. **56** is a schematic diagram of a positioning assembly control module within the control system according to embodiments of the present disclosure;

FIG. **57** is a schematic diagram of a biological indicator (BI) bay heater control module within the control system according to embodiments of the present disclosure;

FIG. **58** is a schematic diagram of a biological indicator (BI) bay door and handler control module within the control system according to embodiments of the present disclosure;

FIG. **59** is a schematic diagram of a camera control module within the control system according to embodiments of the present disclosure;

FIG. **60** is a schematic diagram of an excitation control module within the control system according to embodiments of the present disclosure; and

FIG. **61** is a schematic diagram of a user interface control module within the control system according to embodiments of the present disclosure.

DETAILED DESCRIPTION

According to embodiments of the present disclosure, biological indicator readers, methods and systems provide accurate determinations of sterilization efficacy within a fraction of the time currently needed using conventional tools and methods. For example, while many conventional sterilization efficacy technologies require 24 hours or longer to provide an indication as to whether a sterilization run was successful, the BI readers, systems and methods according to embodiments of the present disclosure can return an efficacy determination within only several minutes. This represents a dramatic improvement over conventional sterilization efficacy technologies, and allows the equipment subjected to the tested sterilization procedure to be used much sooner than would otherwise be possible using current sterilization efficacy testing technology.

Embodiments of the present disclosure are directed to a system for determining the efficacy of a sterilization process

(also referred to herein, interchangeably, as a "sterilization run"). Throughout this disclosure and the accompanying claims, "determining the efficacy of a sterilization process" is used interchangeably with the phrase "sterility assurance," and both terms refer to the same thing, i.e., assessing whether a sterilization process (or run) was successful (e.g., in killing the bacterial spores inside a biological indicator). Aspects of embodiments of the present disclosure are directed to a biological indicator (or "bioindicator" or "BI") **100**, a process challenge device (also referred to herein, interchangeably, as a "PCD") **200**, and a bioindicator reader (also referred to herein, interchangeably, as a "biological indicator reader" or "BI reader") **300**. Aspects of embodiments of the present disclosure are further directed to a method of determining sterilization efficacy utilizing the biological indicator **100** and/or the PCD **200**, and the BI reader **300**. For example, in some aspects of embodiments of the present disclosure, the method may include subjecting the BI **100** and/or the PCD **200** to a sterilization procedure (or sterilization run), and after completing the sterilization run, inserting the biological indicator **100** into the BI reader **300**, which BI reader **300** then tests the biological indicator **100** to determine whether the sterilization run to which the BI was exposed was effective.

Referring to FIGS. **1-12**, according to example embodiments, the biological indicator **100** includes a BI housing **110**, a germinant container **160**, a germinant releaser **170**, a spore carrier **180**, and an imaging window **190**. The BI housing **110** houses the germinant container **160**, the germinant releaser **170**, and the spore carrier **180**. The imaging window **190** allows for imaging of spore activity on the spore carrier **180** by an optical assembly of the BI reader **300**, as discussed in greater detail below.

The BI housing **110** is not particularly limited, and may have any suitable shape such that the BI housing **110** may house the germinant container **160**, the germinant releaser **170**, and the spore carrier **180**, and such that the BI housing **110** may be received by the BI reader **300** and, in some embodiments, such that the BI housing **110** may be received by the the PCD **200**, as discussed further below. According to embodiments, for example, the BI housing **110** has a substantially obround shape (or stadium shape) in a plan view, and has a BI length L_{BI} along a length direction Y_{BI} thereof that is greater than a BI width W_{BI} along a width direction X_{BI} thereof. The BI length L_{BI} and BI width W_{BI} are not particularly limited, but may be selected to fit within the BI reader **300**. For example, in some embodiments, the BI length L_{BI} may be selected such that a user may relatively easily grip the biological indicator **100** at a second end **100***b* thereof to facilitate insertion of an opposite first end **100***a* of the biological indicator **100** into the BI reader **300**. In some embodiments, for example, the BI length L_{BI} may be approximately 2 to 4 times greater than the BI width W_{BI}, for example about 2 to 3 times greater, about 2.5 to 3 times greater, about 2.6 to about 2.9 times greater, or about 2.75 to about 2.8 times greater than the BI width W_{BI}.

Referring to FIG. **1**, the BI housing **110** may include a first shell (e.g., an upper portion or an upper shell) **120** and a second shell (e.g., a lower shell or a lower portion) **130** that mate together to form the BI housing **110**. However, the present disclosure is not limited thereto, and the BI housing **110** may be formed integrally, for example, so long as the contents housed inside of the BI housing **110** can be safely and securely inserted inside the BI housing **110**, or the BI housing **110** may be formed of additional components.

In embodiments including mated first and second shells **120** and **130**, the configuration and mating profile of the first and second shells **120** and **130** are also not particularly limited, and may be any such configuration or mating profile suitable to securely enclose the contents housed within the BI housing **110**. For example, in some embodiments, the first and second shells **120** and **130** may be mated generally along a periphery **115** of the BI housing **110**. The periphery **115** may generally equally bisect the thickness of the BI housing. However, in some embodiments, as shown generally in FIGS. **1** and **2**, the periphery **115** may be skewed or diagonal relative to the thickness dimension of the BI housing, creating a thinner end **130***a* and a thicker end **130***b* of the second (or lower) shell (as shown, e.g., in FIG. **6**).

The material of the BI housing **110** is not particularly limited, and may be any material capable of withstanding the sterilization conditions it will be exposed to during the tested sterilization run (e.g., autoclave conditions) and that can safely and securely house the contents of the BI housing **110**. Some non-limiting examples for such a material for the BI housing **110** include polypropylene homopolymers, and the like.

Referring to FIG. **3**, according to embodiments, the first shell **120** has a grip portion **120***b* at the second end **100***b* and extending toward the first end **100***a*, and a protrusion portion **120***a* at the first end **100***a* that protrudes from the grip portion **120***b* in a thickness direction Z_{BI} of the biological indicator **100** (e.g., the protrusion portion **120***a* protrudes away from the second shell **130** when the BI housing **110** is assembled). In some embodiments, when viewed in a plan view, the protrusion portion **120***a* may have a substantially circular shape, but this disclosure is not limited thereto, and the protrusion portion may have any suitable shape such that the BI **100** fits within the BI reader **300**. Also, the diameter (or other dimensions) of the protrusion portion **120***a* may generally correspond to (or be equal to) the BI width W_{BI}, but again the present disclosure is not limited thereto, and the protrusion portion **120***a* may have any suitable dimensions (including those that may extend beyond the BI width W_{BI}) so long as the BI fits within the reader. As discussed further below, the protrusion portion **120***a* (together with the corresponding portion of the second shell **130**) defines a cavity inside the BI housing **110** where the germinant releaser **170**, at least a portion of the germinant container **160**, and the spore carrier **180** are housed.

According to embodiments, the protrusion portion **120***a* may define an opening (e.g., a through hole) **121** that is configured to receive a germinant release lever **401** in the BI reader **300**. The opening **121** allows for rupture of the germinant container **160** when the germinant release lever **401** is actuated, as discussed further below. According to embodiments, the opening **121** may be sealed to prevent sterilant entry prior to BI activation. Any suitable sealant material may be used for this purpose, and one non-limiting example of such a sealant includes a foil sealant. Upon activation of the BI, the germinant release lever **401** will break the seal during entry into the opening **121**. However, the opening **121** may also remain open (i.e., the seal may be omitted) to allow sterilant to enter the BI housing **110** when the biological indicator **300** is placed in an autoclave chamber, or other sterilization chamber. As shown in FIGS. **1**, **3** and **4**, the opening **121** is positioned generally at the center of the protrusion portion **120***a*, but this disclosure is not limited thereto. Indeed, the opening **121** may be positioned anywhere on the protrusion portion so long as the germinant release lever **401** of the BI reader **300** can enter the opening upon actuation, and so long as the position of the opening

121 allows actuation of the germinant release lever **401** to rupture the germinant container **160**, as discussed further below.

According to embodiments, the opening **121** may be sealed, for example heat sealed with foil (as discussed above), to prevent sterilant from entering through the opening **121**. In such embodiments, the BI housing **110** may include a sterilant opening **121'** (see FIG. **6**) that is separate from the opening **121** and that provides an alternate (or additional) route for the sterilant (e.g., steam) to enter the BI housing **110** during sterilization. The sterilant opening **121'** may be positioned in any suitable location on the BI housing **110**, including on either the first or second shell **120** or **130**. In some embodiments, for example, the sterilant opening **121'** may be a through-hole defined in the second end **100***b* of the BI housing **110**, e.g., in the second shell **130** (as shown in FIG. **6**). In some embodiments, the sterilant opening **121'** may be a through-hole defined in an indentation **137***a* in the second shell **130**, as discussed further below (see FIG. **14**). Additionally, while the sterilant opening **121'** is discussed here in connection with embodiments in which the opening **121** is sealed against sterilant entry, in some embodiments, the BI may have both an unsealed opening **121** (which allows for sterilant entry) as well as the sterilant opening **121'** (which provides as additional avenue for sterilant entry).

According to embodiments, the first shell **120** may further include a visual indicator **122**, for example, an arrow or a triangle, which points toward the first end **100***a* that corresponds to an insertion direction of the biological indicator **100** into the BI reader **300**. The grip portion **120***b* may include a label portion **123** that is configured to receive a label **126** (e.g., a sticker) (see, e.g., FIG. **12**) for easily marking and/or labeling the biological indicator **100**. The label portion **123** may also have a substantially obround shape with a smaller diameter, but the present disclosure is not limited thereto, and the label portion **123** may have any suitable shape such that a user can add identification information to a surface of the grip portion **120***b*. According to embodiments, the label portion **123** is untextured (e.g., smooth) such that a sticker may be easily applied and/or removed, and/or such that a user can easily write directly onto the label portion **123**. And in some embodiments, the label portion **123** is defined by a recessed portion (or indentation) in the surface of the first shell (as shown generally in FIG. **1**). However, it is understood that the label portion **123** may simply be a portion of the surface of the grip portion **120***a* of the first shell **120**, and may not be defined by a visually discernible artifact or disruption in the first shell **120** surface (i.e., the surface of the grip portion **120***a* of the first shell **120** may be substantially continuous and smooth).

Referring to FIG. **4**, according to some embodiments, when the BI housing **120** is assembled, a lower edge of the first shell **120** may be angled relative to the length direction Y_{BI}. For example, the top surface of the first shell **120** may form an angle θ_{BI} relative to the length direction Y_{BI}, such that at least a portion of the top surface of the first shell **120** is not parallel to the length direction Y_{BI}. In some embodiments, the angle θ_{BI} relative to the length direction Y_{BI} may be created by the thicker and thinner ends **130***a* and **130***b* of the second shell **130**, as discussed generally above and in more detail below. In such embodiments, the first shell **120** considered on its own (unmated with the second shell) may have a substantially parallel profile with respect to the length direction Y_{BI}, but obtains a non-parallel (or slanted or diagonal) profile when assembled with (or mated to) the second shell.

According to example embodiments, an inner surface **124** of the first shell **120** may include one or more (or in some embodiments, a plurality of) grooves **125** along its periphery that are configured to mate (e.g., securely mate) with corresponding protrusions **139** on a periphery of the second shell **130**. However, the mating configuration of the first and second shells **120** and **130** are not limited to this interaction of grooves **125** and protrusions **139**, and may instead be any configuration suitable for securely closing the BI housing **110** in a manner that will withstand the conditions of the sterilization process to which it is intended to be exposed. For example, any suitable snap-fit, friction fit, or interference fit engagement between the first and second shells may be used, or the first and second shells may be more fixedly attached to each other, e.g., by an adhesive, or the like.

Referring to FIGS. **5-7**, according to embodiments, the second shell **130** also has a substantially obround shape when viewed in a plan view. A bottom **131** of the second shell **130** defines a bottom opening (e.g., a through hole) **132**, which receives the imaging window **190**. The bottom opening **132** is formed in an area of the first end **100***a* of the biological indicator **100**. According to embodiments, when the first shell **120** and the second shell **130** of the BI housing **110** are mated with each other, a center C of the bottom opening **132** is aligned with (e.g., stacked beneath) the opening **121** along the thickness direction Z_{BI}. However, it is understood, that the bottom opening **132** is not limited thereto, and may be positioned anywhere on the second shell **130** such that it can receive the imaging window and such that the BI reader **300** can image the spores through the imaging window.

According to embodiments, the bottom opening **132** may have an "Odin's cross" shape, as illustrated in FIGS. **5** and **7**. For example, the bottom opening **132** may have a circular portion, with a plurality of protrusions extending from the circular portion, for example four protrusions extending beyond the circular portion in an equilateral cross-shape. However, embodiments of the present disclosure are not limited thereto, and the bottom opening **132** may have any suitable shape. The example Odin's cross shape of the bottom opening **132** may reduce the likelihood of bulging of the spore carrier **180** by allowing air to pass through the protrusion regions, thereby maintaining an equal (or substantially equal) pressure on opposing sides of the spore carrier **180**.

Referring to FIG. **7**, the bottom **131** of the second shell **130** further includes a window notch **133** that surrounds the bottom opening **132** and is configured to receive the imaging window **190** therein.

According to some embodiments, the imaging window is transparent, such that the bottom opening **132** may remain visible to be used to assist in determining proper alignment of the biological indicator **100** when it is inserted into the BI reader **300**. The imaging window **190** may be any suitable material without limitation. Some nonlimiting examples of suitable such materials include thermoplastic polymers, e.g., polymethylpentene, and the like. According to embodiments, the biological indicator **100** may further include a retaining ring **191** which holds the imaging window **190** in the bottom opening **132**. The retaining ring **191** may be made of any suitable material without limitation, a nonlimiting example of which includes Aluminum **6061**. The window notch **133** may have a circular shape, for example, such that the imaging window **190** and the retaining ring **191**

may be inserted into the window notch **133** with relative ease. However, the present disclosure is not limited thereto, and the window notch **133** may have any suitable shape. The retaining ring **191** may seal the imaging window **190** to the window notch **133**, for example, without creating a hermetic seal but while still preventing airborne organisms from entering the BI housing **110** through the bottom opening **132**.

According to embodiments, the second shell **130** may further include a channel **134** which holds the germinant container **160**. For example, the channel **134** may be formed near a center of the biological indicator **100** and may have an open end that faces the first end **100***a* of the biological indicator **100**. However, the position of the channel is not limited to this, and may be placed anywhere else in the second shell that is suitable for holding the germinant container **160**. In some embodiments, the channel **134** may be defined by a channel wall **135** having a substantially U-shape when viewed in a plan view, which extends away from the bottom **131** of the second shell **130** in the thickness direction Z_{BI}. In some embodiments, the channel wall **135** may be formed by creating a pair of grooves extending from the bottom **131**, as can be seen in FIG. **7**, for example. The channel wall **135** may include one or more connecting portions **135***a*, which connect the U-shaped channel wall **135** to a side wall **136** of the second shell **130**, as illustrated in FIG. **5**. In some embodiments, the second shell **130** may include a plurality of connecting portions **135***a* to enhance stability of the channel wall **135**. A channel bottom surface **135***b* may have a shape that substantially corresponds to a shape of the germinant container **160**. For example, the channel bottom surface **135***b* may have a rounded shape or a chamfered shape which accommodates the germinant container **160**, which may have a rounded vial shape. The channel bottom surface **135***b* may also have a varying thickness, such that the channel bottom surface **135***b* slopes toward the first end **100***a* of the biological indicator **100** (see, e.g., FIG. **6**).

According to embodiments, the channel wall **135** is angled, which receives the germinant container **160**. As such, the germinant **165** may flow downwardly through gravitational forces, further facilitating contact between the germinant **165** and the germinant pad **185**.

According to embodiments, the second shell **130** may further include a projection **137** at an area of the second end **100***b* of the biological indicator **100**, located between the side wall **136** and the channel wall **135** along the length direction Y_{BI}. The projection **137** may have a circular shape with a diameter that is slightly less than the width W_{BI} of the biological indicator **100**, thereby forming the indentation **137***a* in an outer surface of the bottom **131** of the second shell **130**. However, the present disclosure is not limited thereto, and the projection **137** may have any suitable shape and/or may be omitted. According to some embodiments, the indentation **137***a* may be sized to receive a process indicator **137***b* that indicates whether the biological indicator **100** has been exposed to a sterilant.

The second shell **130** further includes a side wall **136** extending from the bottom **131** in the thickness direction Z_{BI}. An outward facing surface of the side wall **136** may include an insertion groove **138** at the first end **100***a* and having a substantially U-shape. The insertion groove **138** is configured to mate with a BI bay **375** and/or a BI latch **384** of the BI reader **300** to facilitate proper insertion of the biological indicator **100** into the BI reader **300**. The insertion groove **138** may also include insertion projections **138***a* at opposite sides of the insertion groove **138** near respective ends of the insertion groove **138**, which each define an insertion notch **138***b* at respective ends of the insertion groove **138**, as illustrated in FIG. **2**. The insertion projections **138***a* allow for the BI latch **384** to securely hold the biological indicator **100** in place after insertion into the BI bay **375** of the BI reader **300**, for example, by defining the insertion notches **138***b* which receive a rib **387** of the BI latch **384**, and inhibiting removal of the biological indicator **100** while the BI latch **384** is in contact with the biological indicator **100**. The insertion groove **138** may wrap around the first end **100***a* of the biological indicator **100**, and may be symmetrical on both sides of the biological indicator **100**, though the present disclosure is not limited thereto. According to embodiments, the biological indicator **100** may include the insertion notch **138***b* and the insertion projection **138***a* at only one side of the insertion groove **138**.

The second shell **130** may further include the protrusions **139** at the outer surface of the side wall **136**, which are configured to securely mate with the grooves **125** of the first shell **120**. It will be appreciated that, according to embodiments, the grooves **125** may be formed in the second shell **130** and the protrusions **139** may be formed in the first shell **120**. Moreover, other means for securely fastening the first shell **120** and the second shell **130** may be used, as are known in the art, and discussed generally above. It will also be appreciated that an upper edge of at least a portion of the side wall **136** may be formed at an angle that is inversely equal to the angle θ_{BI}. In other words, at least a portion of the side wall **136** may be formed at the angle θ_{BI} below the length direction Y_{BI} such that the first shell **120** and the second shell **130** snugly mate with each other (see, e.g., FIGS. **6** and **2**).

According to embodiments, the biological indicator **100** may further include a germinant releaser support **140**, which is housed inside the BI housing **110**, for example, near the first end **100***a* of the biological indicator **100**, and below the protrusion portion **120***a* of the first shell **120**. The germinant releaser support **140** houses (or accommodates) the germinant releaser **170** and is configured to bring the germinant releaser **170** into contact with the germinant container **160**, for example, by application of force in the thickness direction Z_{BI}. According to an example embodiment, the germinant releaser support **140** may have a saddle shape.

Referring to FIGS. **8-11**, according to some embodiments, the germinant releaser support **140** may include a seat **141**, a plurality of support legs **142**, a center leg **143**, a germinant releaser opening **144**, and a tab **155**. The seat **141** may have a substantially semicircular shape when viewed in a plan view, with a rounded portion facing the first end **100***a* of the biological indicator **100**. According to embodiments, a width of the seat **141** along the width direction X_{BI} is less than the BI width W_{BI}. As such, the germinant releaser support **140** may easily be installed in the BI housing **110** without interference with the BI housing **110**.

The support legs **142** may each include an extension portion **142***a* that extends away from the seat **141** along the length direction Y_{BI} toward the second end **100***b*, and a projection portion **142***b* that extends from an end of the extension portion **142***a* opposite to the seat **141**, and extends downwardly in the thickness direction Z_{BI}. The support legs **142** may be formed at opposite ends of the seat **141** along the width direction X_{BI}, such that the support legs **142** straddle the channel **134** and the germinant container **160** when the biological indicator **100** is assembled. In addition, the support legs **142** may be offset from an upper surface **141***a* of the seat **141** in the thickness direction Z_{BI}. The projection portions **142***b* are configured to extend past ones of the

connecting portions **135***a* when the germinant releaser support **140** is inserted into the BI housing **110**, thereby maintaining the relative placement of the germinant releaser support **140**. According to embodiments, the support legs **142** are located at a height on the seat **141** such that the extension portions **142***a* may rest on an upper surface of the connecting portions **135***a*. As discussed above, this configuration allows for relatively easy placement and alignment of the germinant releaser support **140**, without requiring a clearance fit or a tight fit, which can cause issues and delays during production, and which would limit flexibility of the germinant releaser support **140** when a downward force is applied to the germinant releaser support **140**.

The center leg **143** may include a center leg extension portion **143***a* and a center leg projection portion **143***b*. The center leg **143** may be located at a generally central portion of the seat **141** along the width direction X_{BI} such that the center leg **143** is located above the channel **134** and the germinant container **160** when the biological indicator **100** is assembled. However, the present disclosure is not limited to this, and the center leg **143** may be positioned anywhere on the germinant releaser support **140** so long as the center leg **143** remains capable of contacting the germinant container **160**, as discussed further below. The center leg extension portion **143***a* may extend away from the seat **141** along the length direction Y_{BI}, and may have a length in the length direction Y_{BI} that is less than a length of the support legs **142** along the length direction Y_{BI}. The center leg **143** is configured to be positioned above the germinant container **160** when the germinant container **160** and the germinant releaser support **140** are inside the BI housing **110**. The center leg projection portion **143***b* extends downwardly in the thickness direction Z_{BI}, and is configured to contact the germinant container **160** when force is applied to the germinant releaser support **140** (e.g., upon actuation of the germinant release lever **401** of the BI reader **300**), acting as a spring to concentrate the downward force of the germinant releaser **170** onto the germinant container **160**, as discussed further below.

The germinant releaser support **140** may be made of any suitable material such that the support legs **142** allow for flexible movement of the germinant releaser support **140** along the thickness direction Z_{BI}. For example, the germinant releaser support **140** may be formed of a polymeric material (nonlimiting examples of which include polypropylenes, and the like), which has sufficient give to allow for movement of the seat **141** when downward pressure is applied (along the thickness direction Z_{BI}), but sufficient strength to maintain the support legs **142** in their position relative to the channel **134**.

According to embodiments, the germinant releaser support **140** further includes a tab **145** which protrudes downwardly from the seat **141**. When the BI is in the non-activated state, the center leg projection portion **143***b* and the tab **145** are spaced vertically from the surface of the germinant container **160**. As discussed above, when the BI is activated (i.e., upon actuation of the germinant release lever **401** of the BI reader **300**), the force applied by the germinant release lever **401** overcomes the spring force of the support legs **142**, which, in turn causes the center leg projection portion **143***b* and the tab **145** to come into contact with the germinant container **160**. Upon this contact, each of the center leg projection portion **143***b* and tab **145** act as a spring to concentrate the downward force of the germinant releaser **170** onto the germinant container **160** (e.g., across a diameter of the germinant container).

The seat **141** further defines a germinant releaser opening **144** that is configured to receive the germinant releaser **170** and to maintain positioning between the germinant releaser **170** and the germinant releaser support **140**. For example, the germinant releaser opening **144** may have a substantially cylindrical shape with a length along the width direction X_{BI}. According to embodiments, the length of the germinant releaser opening **144** is greater than a width of the germinant container **160** along the width direction X_{BI} to ensure that the germinant releaser **170** contacts the germinant container **160** upon actuation of the germinant release lever **401** of the BI reader (discussed further below). The germinant releaser opening **144** may include one or more (or a plurality of) stops **146** extending toward each other along the length direction of the germinant releaser opening **144**. The stops **146** serve to prevent the germinant releaser **170** from exiting the germinant releaser opening **144** above the seat **141** when downward pressure is applied to the germinant releaser support **140**. Stated differently, the stops **146** serve to maintain the germinant releaser **170** in the germinant releaser opening **144** upon actuation of the germinant release lever **401** of the BI reader **300** (discussed further below), which ensures that the germinant releaser **170** contacts the germinant container **160** with enough force to rupture or break the germinant container **160**.

After the biological indicator **100** is inserted into the BI bay **375**, the germinant release lever **401** is activated, causing it to extend into the opening **121** of the biological indicator **100** and apply downward pressure onto the components inside of the biological indicator **100**. More specifically, the germinant release lever **401** presses downwardly onto the germinant releaser support **140** (directly or via the sterilant membrane **105**), which presses downwardly toward the bottom **131**. The germinant releaser support **140** flexes downwardly, bringing the germinant releaser **170** into contact with the germinant container **160**, thereby rupturing the germinant container **160** and releasing the germinant **165** into the BI housing **110**. The germinant **165** flows downwardly toward a germinant pad **185**, which captures (e.g., absorbs) the germinant **165**, directing (e.g., wicking) the germinant **165** through the germinant pad toward the spore carrier **180**. If the sterilization process was successful, the spores **181** on the spore carrier **180** were killed during the sterilization process, at which point the spores released DPA. The DPA from these dead spores may be bound by the photoluminescent component of the germinant and generate a static background level of DPA that is detected by the BI reader **300**. However, if any of the spores on the spore carrier remain viable after completion of the sterilization process, those spores will germinate upon contact with the germinant compound, and will release DPA upon germination. Once the DPA is released from these viable spores, the DPA will be bound by the photoluminescent component, and detected by the BI reader **300** as a DPA signal above the static background level (when such a background signal is present). This detection and distinction between DPA signals is discussed in further detail below.

According to some embodiments, the biological indicator **100** may further include the germinant pad **185**. The germinant pad **185** may be a wicking layer that is located below the germinant container **160**. The germinant pad **185** may include any material capable of wicking a germinant (e.g., a germinant fluid) **165** that is expelled from the germinant container **160** after the germinant container **160** is ruptured. Nonlimiting examples of suitable such wicking materials include cotton and cellulose-based materials, and any other wicking materials known to those of ordinary skill in the art.

Upon rupture of the germinant container **160**, the germinant **165** released from the germinant container **160** transports (or wicks) through the germinant pad **185** to a spore carrier **180** located below the germinant pad **185**. The wicking (or transporting) function of the germinant pad **185** is generally provided by the material of the germinant pad **185**, which as noted generally above, may be any material suitable for wicking or transporting a fluid having the composition and properties of the germinant solution, e.g., by capillary-like action. The germinant pad **185**, therefore, provides a relatively controlled delivery of the germinant **165** through the germinant pad **185** to the spore carrier **180**.

The germinant pad **185** may have any suitable shape and size without limitation so long as it is capable of transporting the germinant **165** through the pad to the spore carrier **180**. In some embodiments, for example, as shown in FIG. **12**, the germinant pad **185** may have a generally rectangular shape. As shown, the germinant pad **185** may have an area (i.e., width×length) greater than the area of the spore carrier **180** to ensure that the germinant **165** is delivered efficiently and in sufficient amount to the spore carrier **185**. Additionally, in some embodiments, the greater area of the germinant pad **185** allows the germinant pad to maintain any rogue pieces of the broken germinant container **160** and keep those pieces from contaminating the spore carrier **180**. In furtherance of that end, in some embodiments, the germinant pad **185** may also include a protrusion from a generally rectangular main body, which protrusion is configured to fit in the channel **134** holding the germinant container **160**. And in embodiments in which the germinant pad **185** is not generally rectangular in shape, the germinant pad **185** may have any other shape with at least a portion extending into the channel **134**.

The spore carrier **180** may include any support material capable of housing bacterial spores **181**. The spores **181** may be any bacterial spores **181** suitable for use to determine the efficacy of a sterilization process. The bacterial spores selected to determine the efficacy of sterilization may differ depending on the type of sterilization process being tested. In general, highly resistant bacterial species are selected since these species are particularly difficult to kill, and therefore provide a more accurate assessment of sterilization efficacy. Traditionally, bacteria of the genera *Geobacillus* and *Bacillus* have been used due to their high resistance to sterilization, e.g., steam sterilization. Accordingly, the spores **181** on the spore carrier **180** may include a bacteria from these genera, but the present disclosure is not limited thereto, and any bacterial spores known for use in determining sterilization efficacy may be used without limitation, e.g., those of the genus *Clostridium*.

The spores **181** may be applied to the spore carrier **180** by any suitable means and methods, without limitation. According to embodiments, for example, the bacteria may be suspended in an alcohol (e.g., ethanol or 40% ethanol), and the spores **181** may include a spore population of between about 1.0×10^7 spores/0.1 ml to about 3.0×10^7 spores/0.1 ml. The spores **181** may have a D-Value Range of between about 1.9 to about 2.1 minute D-Value at 121 C steam. According to embodiments, approximately 200,000 spores **181** may be applied to the spore carrier **180**, and in some embodiment, at least 100,000 spores **181** are applied to the spore carrier **180**. According to embodiments, the spores **181** are applied to a bottom surface of the spore carrier **180** (or a surface of the spore carrier **180** facing the imaging window **190**) so that the germinant **165** reaches the spores **181** after saturating the spore carrier **180**. This prevents the flow of germinant **165** from oversaturating the spores **181**, which may affect the readings by the BI reader **300**.

The spore carrier **180** may be formed of any suitable material with sufficient porosity and density such that the spores **181** do not pass through the spore carrier **180**, and such that the spore carrier **180** withstands the high temperatures encountered during the sterility procedure (e.g., an autoclave procedure). For example, the spore carrier **180** may have a pore size of approximately 0.1 to about 0.8 μm, about 0.2 to about 0.4 μm, or about 0.3 μm. According to embodiments, the spore carrier **180** may have a gray or black color to enable improved background correction during testing of the biological indicator **100**, as discussed further below. Any suitable dye may be used to color the spore carrier **180** gray or black so long as the dye is not cytotoxic. Non-limiting examples of suitable spore carrier materials include cellophane-based materials, such as poly-cellophane materials, polyester materials (such as, e.g., polyethylene terephthalate), and the like.

Any of the spores **181** that were killed during the sterilization procedure released dipicolinic acid (DPA). The DPA released by these dead spores **181** may diffuse into a background DPA level that may be detected via an optical assembly of the BI reader **300** (discussed further below). In some embodiments, if the early DPA readings by the BI reader match expected levels based on the known bacterial spore population on the carrier, this provides an early indication that the spores inside the BI were sufficiently exposed to the sterilant during the sterilization procedure. Conversely, if the early DPA readings show an absence of DPA or DPA releases lower than the anticipated threshold, this may indicate that the sterilization process failed, or that the spores inside the BI were not sufficiently exposed to the sterilant. If any of the spores **181** remain viable after sterilization, the viable spores **181** will germinate upon exposure to the germinant **165** and release their DPA, resulting in time-lapsed DPA spikes indicative of spore germination (and thus spore survival) and sterilization failure. This is discussed in further detail below.

The shape and size of the spore carrier **180** is not particularly limited, and may be any shape and size suitable to hold the population of bacterial spores **181**. However, in some embodiments, the spore carrier is not larger than the imaging window **190** so that the entire spore carrier can be imaged by the BI reader **300** and analyzed on a pixel-by-pixel basis, as discussed further below. According to some embodiments, for example, the spore carrier **180** may have a disc shape that generally corresponds in size and shape to the imaging window **190**. According to embodiments, the spores **181** are deposited on the spore carrier **180** such that the spores **181** are centered in the bottom opening **132** so that an optical assembly of the BI reader **300** may be aligned to a center of the bottom opening **132** (and therefore to a location of the spores **181**). The spores **181** are deposited on the spore carrier **180** according to any suitable method. For example, the spores **181** may be deposited on the spore carrier **180** while suspended in a liquid and by applying a vacuum to extract fluid during deposition of the spores **181**, thereby creating a dry deposition of the spores **181** on the spore carrier **180**. As such, the likelihood of the spores **181** moving on the spore carrier **180** after deposition is reduced. According to some embodiments, the spore carrier **180** may be pre-treated to improve hydrophilicity. As such, the germinant solution **165** may be more effectively transported to the spores **181**, and the likelihood of imaging artifacts may be reduced. Examples of suitable hydrophilicity treatments include UV exposure, plasma oxygen, or the like, but the present disclosure is not limited thereto.

As noted generally above, the germinant container **160** houses a germinant (or germinant solution or liquid) **165**. The material and construction of the germinant container **160** is not particularly limited so long as it can hold the germinant solution **165**, withstand the conditions of the sterilization process (e.g., the high heat and steam of an autoclave), and can be broken or ruptured by the germinant releaser **170** upon actuation by the reader **300**. Those of ordinary skill in the art would be capable of selecting an appropriate such material, but one non-limiting example includes glass.

According to some embodiments, the germinant container **160** may be an ampule (or ampoule) made of glass. The germinant container **160** has any suitable thickness such that the germinant container **160** contains the germinant **165** during the sterilization cycle (e.g., an autoclave cycle), and that the germinant container **160** ruptures when pressure is applied to the germinant container **160** by the germinant releaser **170**. According to one or more embodiments, the germinant releaser **170** may be a dowel comprising metal, ceramic, or the like, though the present disclosure is not limited thereto. The germinant releaser **170** (e.g., as a dowel) may have a length in the width direction X_{BI} that is greater than a width of the germinant container **160** in the width direction X_{BI} to increase the likelihood that the germinant releaser **170** ruptures the germinant container **160**. According to example embodiments, the germinant releaser **170** may have a spherical shape (such as a BB), or any other suitable shape and density that allows for rupture of the germinant container **160**.

According to embodiments, the biological indicator **100** may further include a gauze or other wrap provided around the germinant container **160**, which helps collect broken pieces of the germinant container **160** (e.g., glass pieces of the ampule) that are created by rupturing the germinant container **160**.

The germinant solution **165** is housed inside the germinant container **160** such that the germinant solution **165** is not exposed to the sterilization conditions of the sterilization process (e.g., is not exposed to the steam produced in an autoclave). The germinant solution contains at least a germinant compound and a photoluminescent component, and may further contain a solvent, e.g., water. According to embodiments, a surfactant, such as sodium dodecyl sulfate (SDS) may be added to the germinant solution **165**, which further improves hydrophilicity of the spore carrier **180** upon exposure to the germinant solution **165**. The germinant compound is not particularly limited, and may be any compound capable of inducing germination of the bacterial spores **181** carried on the spore carrier **180**. Those of ordinary skill in the art would be capable of selecting an appropriate such germinant compound, e.g., based on the type of bacterial spores carried on the spore carrier. Non-limiting examples of suitable germinants includes L-alanine, potassium combined with one or more simple sugars, and a combination of valine and isoleucine.

The photoluminescent component is also not particularly limited, but should be a component suitable to cause or enhance the photoluminescence of the DPA expelled by the bacterial spores in the visible light range, thereby improving the detectability of released DPA by the BI reader **300**. Non-limiting examples of suitable such components include lanthanide complexes, e.g., complexes including a lanthanide ion and a counter-ion. As would be understood by those of ordinary skill in the art, "lanthanides" encompass elements **57-71** of the periodic chart, i.e., La, Ce, Pr, Nd, Pm, Sm, Eu, Gb, Tb, Dy, Ho, Er, Tm, Yb, and Lu. In some embodiments, the lanthanide ion of the photoluminescent compounds may include La, Ce, Eu or Tb, for example, Eu or Tb, and in some embodiments, the lanthanide ion may be Tb. Those of ordinary skill in the art are capable of selecting an appropriate anion for the lanthanide complex, but some nonlimiting examples include halides (e.g., chlorides, fluorides, bromides or iodides). In some embodiments, for example, the anion may be a chloride. For example, in some embodiments the photoluminescent component includes terbium chloride hexahydrate. It will be appreciated by those of ordinary skill in the art that the methods, systems, and apparatuses, including the germinant solution compositions, disclosed in U.S. Pat. No. 7,306,930 to Ponce et al. titled "Method bacterial endospore quantification using lanthanide dipicolinate luminescence," U.S. Pat. No. 7,608,419 to Ponce titled "Method and apparatus for detecting and quantifying bacterial spores on a surface," U.S. Pat. No. 7,611,862 to Ponce titled "Method and apparatus for detecting and quantifying bacterial spores on a surface," U.S. Pat. No. 9,469,866 to Ponce titled "Method and apparatus for detecting and quantifying bacterial spores on a surface," U.S. patent application Ser. No. 15/283,268, which is currently pending, to Ponce titled "Method and apparatus for detecting and quantifying bacterial spores on a surface," and U.S. Pat. No. 9,816,126 to Ponce titled "Method and apparatus for detecting and quantifying bacterial spores on a surface," U.S. Pat. No. 7,563,615 to Ponce titled "Apparatus and method for automated monitoring of airborne bacterial spores," U.S. patent application Ser. No. 10/355,462 to Ponce et al., now abandoned, titled "Methods and apparatus for assays of bacterial spores," U.S. Pat. No. 8,173,359 to Ponce et al. titled "Methods and apparatus and assays of bacterial spores," U.S. patent application Ser. No. 13/437,899 to Ponce et al., now abandoned, titled "Methods and apparatus for assays of bacterial spores," U.S. Pat. No. 10,612,067 to Ponce et al. titled "Methods and apparatus for assays of bacterial spores," U.S. patent application Ser. No. 16/841,534 to Ponce et al. titled "Methods and apparatus for assays of bacterial spores," each of which is incorporated herein by reference in its entirety, may also be utilized.

According to some embodiments, the biological indicator **100** may also include a sterilant membrane **105** that is located between the protrusion portion **120***a* of the first shell **120** and the germinant releaser support **140**. The sterilant membrane **105** is sterilant permeable (e.g., steam permeable) to allow the sterilant access to the interior of the BI **100**. The material of the sterilant membrane **105** is not particularly limited so long as it is permeable to the sterilant. Non-limiting examples of suitable sterilant membrane materials include cellulose-based papers and Kraft paper, e.g., 40 pound Kraft paper. The sterilant membrane **105** may have any suitable shape and size, without limitation. In some embodiments, for example, the sterilant membrane may have a generally circular shape, and may be configured to fit inside the protrusion portion **120***a* of the first shell **120**. According to embodiments, the sterilant membrane **105** may be omitted.

According to some embodiments, the biological indicator **100** may further include a secondary spore carrier and secondary spores at a second location separate from the spore carrier **180**. The secondary spores are also exposed to the sterilant when the biological indicator **100** undergoes a sterilization process. However, unlike the spores **181** on the spore carrier **180**, the secondary spores are not exposed to the germinant **165** when the biological indicator **100** is activated in the BI reader **300**, and can instead be used in a reference culture test to verify the results obtained from the

BI reader **300**. According to embodiments, the secondary spores may be located outside of the channel wall **135**, e.g., between the channel wall **135** and the side wall **136**.

The biological indicator **100** according to embodiments may be assembled as follows. First, the spore carrier **180** is arranged inside the second shell **130** above the bottom opening **132** and the spores **181** are deposited on the spore carrier **180**. Then, the imaging window **190** is inserted into the window notch **133** of the second shell **130** and is secured in place using the retaining ring **191**. The germinant pad **185** is arranged above the spore carrier **180**. The germinant container **160** is arranged above the germinant pad **185** and in the channel **134**, such that the germinant container **160** rests in the channel **134** and is downwardly angled toward the bottom **131** of the second shell **130**. The germinant releaser **170** is inserted into the germinant releaser opening **144**, typically before insertion of the germinant releaser support **140**. The germinant releaser support **140** is arranged above a portion of the germinant container **160** above the imaging window **190**, such that the extension portions **142***a* of the support legs **142** rest on the connecting portions **135***a*, and the center leg **143** rests on another portion of the germinant container **160**. In some embodiments, the germinant releaser **170** is freestanding, i.e., it is not attached to another component of the BI, and enjoys a certain amount of free-play within the BI. The sterilant membrane **105** is arranged above the germinant releaser support **140**, and the first shell **120** is arranged above the sterilant membrane **105**, such that the protrusion portion **120***a*, the sterilant membrane **105**, the germinant releaser support **140**, the germinant releaser **170**, the germinant container **160**, the spore carrier **180**, and the imaging window **190** are in a stacked configuration (see, e.g., FIG. **12**). The grooves **125** of the first shell **120** and the protrusions **139** of the second shell **130** (or vice versa) are then mated together to securely fasten the BI housing **110**. The process indicator **137***b* may be affixed at the indentation **137***a* before, during, or after assembly of the BI housing **110**, or may be omitted.

FIGS. **13-18** illustrate an alternative biological indicator **100'** including a germinant container (e.g., a sealed germinant reservoir) **160'** seated above a germinant releaser **170'**, both accommodated in a second shell **130'** and which omits the germinant releaser support **140** described above. Various features of the biological indicator **100'** are substantially the same as those described above with reference to the biological indicator **100**. As such, additional descriptions thereof may be omitted.

According to embodiments of the present disclosure, the germinant container **160'** may be seated on the germinant releaser **170'**. The germinant releaser **170'** is configured to puncture a barrier **161'** of the germinant container **160'** when downward pressure is applied to the germinant container **160'**.

The germinant container **160'** may include an outer container **162'** having a hollow interior which houses the germinant **165**. The material of the outer container **162'** is not particularly limited so long as it can withstand the sterilization conditions and securely house the germinant solution **165**. In some embodiments, the germinant container is made of a polymeric material, nonlimiting examples of which include polypropylene homopolymers. The outer container **162'** is sealed by the barrier **161'**, for example, an aluminum foil, that may be heat-sealed to a bottom of the outer container **162'**. The barrier **161'** is sufficiently robust to eliminate the risk of friction erosion at the interface of the barrier **161'** and releaser protrusions **171'** of the germinant releaser **170'**, discussed further below.

In a normal or unactivated state (i.e., when the germinant container **160'** is not depressed by the germinant release lever **401**), there may be a gap (e.g., about a 1 mm gap) between an interior surface of the first shell **110** and a top **163'** of the outer container **162'**. The gap may allow for transverse movement of the germinant container **160'** within the BI housing **110**. The top **163'** of the outer container **162'** may have a plurality of radial sterilant release pathways (e.g., radial steam release channels) **164'** that aid the flow of sterilant toward an interior of the BI housing **310** when the biological indicator **100** is undergoing sterilization. The sterilant release pathways **164'** may also prevent the sterilant membrane **105** from collapsing flat against the top **163'** of the germinant container **160'** and blocking the inflow of sterilant, or reducing the likelihood thereof. The sterilant membrane **105** may be deformable and may increase resistance to the sterilant to limit sterilant access inside of the BI housing **110**.

When the germinant container **160'** is depressed by the germinant release lever **401** of the BI reader **300**, the outer container **162'** of the germinant container **160'** is configured to not deflect under the pressure, and the germinant container **160'** in its entirety is moved vertically (along the thickness direction Z_{BI}) down toward and over the germinant releaser **170'**, which breaks the seal at the barrier **161'** and displaces the germinant **165** under pressure. The pressurized evacuation of the germinant **165** can provide reproducibility and speed of release for operation of the BI reader **300**.

In some embodiments, as generally discussed above, the sterilant opening **121'** may be formed in the indentation **137***a* of the second shell **130'**. For example, the indentation **137***a* may be defined by a short circumferential (or peripheral) sidewall **137***c* of the projection **137**, and the sterilant opening **121'** may be formed in the circumferential (or peripheral) sidewall **137***c* to provide sterilant access into the cavity or interior of the BI housing. The second shell **130'** may further include a substantially cylindrical sidewall **136'** which houses the germinant container **160'** and the germinant releaser **170'**, as illustrated in FIG. **14**.

Referring to FIGS. **17-18**, the germinant releaser **170'** may include a plurality of support legs **173'** (e.g., three support legs **173'**) extending from (e.g., extending radially from) a body portion **172'** of the germinant releaser **170'**. The support legs **173'** may separate the body portion **172'** of the germinant releaser **170'** from the bottom **131** of the second shell **130**. The body portion **172'** includes releaser protrusions **171'**, which protrude upwardly along the thickness direction Z_{BI} and toward the germinant container **160'**. The releaser protrusions **171'** are configured to engage the barrier **161'** at the bottom of the germinant container **160'**. As the germinant container **160'** is depressed toward the body portion **172'**, the releaser protrusions **171'** press against the barrier **161'** and break the seal formed by the barrier **161'**, thereby releasing the germinant **165**. The body portion **172'** may include one or more releaser notches **174'** around a periphery of the body portion **172'** that facilitate flow of the germinant **165** past the germinant releaser **170'** and toward the germinant pad **185** when the barrier **161'** of the germinant container **160'** is ruptured.

According to embodiments, the germinant releaser **170'** is free of any sharp edges or pointed upward facing surfaces, including the releaser protrusions **171'**, so that the germinant container **160'** may safely rest on top of the body portion **172'** by way of gravity without prematurely rupturing (e.g., inadvertently rupturing) the germinant container **160'**.

The material of the germinant releaser is not particularly limited, as discussed generally above in connection with germinant releaser **170**. In some embodiments, for example, the germinant releaser **170'** may be made of a polypropylene homopolymer.

The germinant container **160'** utilizing a sealed foil, for example, may provide for a relatively long shelf life and durability during the sterilization cycle. However, the foil barrier **161'** may fail during a subsequent dry time following the sterility procedure (e.g., autoclave cycle), and the barrier **161'** may separate to some degree from the outer container **162'**. Suitable material selection for the barrier **161'** may reduce the likelihood of separation.

For convenience, reference is made to the biological indicator **100** in the detailed description below. However, it will be appreciated that other embodiments, including the biological indicator **100'**, may be utilized with the process challenge device **200** and the BI reader **300**.

Referring to FIGS. **19-24**, the biological indicator **100** may be inserted into a process challenge device (PCD) **200** prior to being subjected to the sterilization process. In some embodiments, the PCD **200** may include a tray **210**, a closure portion **240**, a sterilant sterilization integrator (or chemical integrator) **250**, and the BI **100**.

According to embodiments, the tray **210** may define a first cavity **220**, a second cavity **230**, and a sterilant access port **215**. The first cavity **220** has a shape corresponding to a shape of the biological indicator **100** (i.e., of the BI housing **110**), and is configured to receive the biological indicator **100** in a "face-down" configuration, i.e., with the first shell **120** facing and contacting the first cavity **220** and the bottom **131** facing away from the first cavity **220**. The second cavity **230** is configured to receive the sterilant sterilization integrator **250**. The first cavity **220** and the second cavity **230** are in fluid communication with each other. In some embodiments, the sterilant access port **215** is located at a central portion of the tray **210** between the first cavity **220** and the second cavity **230**, but the present disclosure is not limited thereto, and the sterilant access port **230** may be located in any suitable position. The sterilant access port **215** is also in fluid communication with the first cavity **220** and the second cavity **230**.

The material of the tray is not particularly limited so long as it can withstand the sterilization conditions to which is subjected. Some non-limiting examples of suitable materials for the tray **210** include polymeric materials with resistance to sterilization conditions, e.g., polypropylenes. Additionally, the material of the tray may be at least partially transparent to allow for visual confirmation of the sterilant sterilization integrator **250** while sealed.

The sterilant sterilization integrator **250** may be used to confirm that desired sterilant sterilization criteria are met during sterilization by visual confirmation through the tray **210**. For example, the sterilant sterilization integrator **250** may be a PROPPER® VAPOR LINE® steam sterilization integrator, model number 26900925 (PROPPER® and VAPOR LINE® are registered trademarks of Propper Manufacturing Company, Inc.). However, the present disclosure is not limited thereto, and any suitable means for providing an indication of sterilant introduction into the PCD may be utilized.

According to embodiments, the closure portion **240** may be a foil sheet or other material that can maintain a firm seal but is also relatively easily ruptured to allow for removal of the biological indicator **100** after the sterilization procedure. The closure portion **240** may be sealed (e.g., heat sealed) to the tray **210** after the sterilant sterilization integrator **250** and the biological indicator **100** are inserted into the tray **210**.

The assembled PCD **200**, including the biological indicator **100**, may be subjected to the sterilization procedure for testing. During the sterilization procedure, sterilant enters the PCD tray **210** via the sterilant access port **215**, and travels through the tray to the BI housing **110** where it enters the BI via the opening **121'**. After the sterilization procedure is completed, the biological indicator **100** may be removed from the PCD **200** (i.e., from the tray **210**) by puncturing or otherwise separating at least a portion of the closure portion **240** from the tray **210**. The biological indicator **100** is then inserted into the BI reader **300** to determine the efficacy of the sterilization procedure, as discussed in greater below.

Referring to FIGS. **25-28**, an alternative tray **210'** of a PCD **200'** is shown. Various features of the alternative PCD are substantially the same as those described above with reference to the PCD **200**. As such, additional descriptions thereof may be omitted.

According to some embodiments, the tray **210'** of the PCD includes a first cavity **220'** and a tab **260'**. As illustrated in FIGS. **25** and **26**, the first cavity **220'** has a shape corresponding to a shape of the biological indicator **100** (i.e., of the BI housing **110**), and is configured to receive the biological indicator **100** in a sideways configuration, as opposed to the face-down configuration of the first cavity **220** of the PCD **200**. The tray **210'** may have a smaller surface area than the tray **210** described above, and therefore may reduce the likelihood of post-processing warpage of the tray **210'**.

According to embodiments, the first cavity **220'** may receive both the biological indicator **100** and the sterilant sterilization integrator **250**. The sterilant sterilization integrator **250** is separated from the first cavity **220'** by the tab **260'** and is held in place by the tab **260'**. The tray **210'** further includes a sterilant access port **215'**, which is formed near a portion of the tray **210'** to which the closure portion **240** attaches (see FIG. **27**).

The assembled PCD **200'**, including the biological indicator **100**, may be subjected to a sterilization procedure for testing. During the sterilization procedure, sterilant enters the PCD tray **210'** via the sterilant access port **215'**, and travels through the tray **210'** to the BI housing **110** where it enters the BI via the opening **121'**. After the sterilization procedure is completed, the biological indicator **100** may be removed from the PCD **200'** (i.e., from the tray **210'**) by puncturing or otherwise separating at least a portion of the closure portion **240** from the tray **210'**. The biological indicator **100** is then inserted into the BI reader **300** to determine the efficacy of the sterilization procedure, as discussed in greater detail below.

According to embodiments of the present disclosure, the BI reader **300** determines the efficacy of a sterilization run by reading the levels of DPA released by the spores housed in the biological indicator **100** over time. The BI reader **300** includes various modular functional subassemblies that are integrated and interconnected within the BI reader **300** to determine the efficacy of a sterilization run. The BI reader **300** may be operated utilizing an external power supply, for example, a DC power supply.

According to embodiments of the present disclosure, the BI reader **300** includes a BI reader housing **301** including a front panel assembly **310** and a rear panel assembly **390**, an optical assembly including a positioning assembly **340** and a camera assembly **360**, and a heater block assembly **370**. Referring to FIG. **29**, the front panel assembly **310** may include a front panel **311** including a display **312**, one or

more access doors **313**, and corresponding access door releases **314**. According to embodiments, the display **312** may be a touch panel display, such as a thin film transistor liquid crystal display module or an OLED display, that is configured to receive user inputs via touch screen and to display information to a user. However, the present disclosure is not limited to such touch panel displays, and may be any display capable of receiving user inputs (e.g., via tactile buttons which may be designed to allow a user to scroll through various menu options), and displaying necessary information (e.g., via a non-touch screen display window). The display **312** is connected to a display control board **315** (see FIG. **32**), which communicates with various other control boards in the BI reader **300** to operate the BI reader **300**, as discussed further below. The control boards of the BI reader **300** are collectively referred to herein as the control system.

Referring to FIGS. **30-31**, the front panel **311** may define one or more door openings **316**, one or more door release openings **317**, and a display opening **322**. The size and shape of the door openings **316** are not particularly limited so long as the BIs **100** fit within the openings and the openings can accommodate the access doors when the Bis **100** are inserted, as discussed further below. For example, in some embodiments, the door openings **316** may have a substantially rectangular shape when viewed in the plane of the front surface **311**A of the front panel **311**, and may have rounded corners.

As illustrated in FIGS. **30-31**, the front panel **311** may include one or more chambers **326** that respectively correspond to and define the one or more door openings **316**, each having a chamber opening **327** in fluid communication with the respective door openings **316**. The chambers **326** each protrude from a back surface (or inner surface) **311**B of the front panel **311** and are configured to guide the biological indicator **100** to the heater block assembly **370** when it is inserted into the door opening **316**, as discussed further below. The chambers **326** may have any suitable shape without limitation. According to embodiments, the door opening **316** may have a height that is greater than a height of the biological indicator **100**. In such embodiments, the chambers **326** may each have an upwardly sloped portion **326**A (seen best in FIG. **34**) that extends from the back surface **311**B at a lower portion of the door opening **316** and that guides the biological indicator **100** toward the chamber opening **327** when the biological indicator **100** is inserted from below the chamber opening **327**. The chambers **326** may similarly each have a downwardly sloped portion **326**B (seen best in FIG. **34**) that extends from the back surface **311**B at an upper portion of the door opening **316** and that helps guide the biological indicator **100** toward the chamber opening **327** when the biological indicator **100** is inserted from above the chamber opening **327**.

The size and shape of the door release openings **317** are also not particularly limited, and may have any suitable size and shape so long as they can receive the corresponding access door releases **314**. For example, in some embodiments, each of the door release openings **317** may have a substantially obround shape and may be located adjacent its corresponding door opening **316** such that each door opening **316** has a corresponding door release opening **317**. In some embodiments, the door release openings **317** may be located beneath their corresponding door openings **316**, but the present disclosure is not limited thereto, and the door release openings may be located anywhere on the front panel **311**. Indeed, in some embodiments, the door release openings **317** may be located on the front panel in positions that do not correspond, or are not adjacent the corresponding door openings. Each of the door release openings **317** may occupy an area on the front panel that is smaller than the area occupied by their corresponding door openings **317**, but the present disclosure is not limited thereto, and the door release openings **316** may have any suitable size and shape, as noted above.

Referring to FIG. **32**, the display **312** is received in the display opening **322**. According to embodiments, the display **312** and the display opening **322** may each have a substantially circular shape when viewed in a plan view. However, the present disclosure is not limited thereto, and the display **312** and the display opening **322** may have any suitable shape such that the display **312** may be received in the display opening **322** and such that the display **312** may receive instructions from the display control board **315** and be visible to a user. For example, in some embodiments, the display **312** and the display opening **322** may have a square, rectangular, ovular or any other geometric shape. The display **312** provides information to a user, such as whether the BI reader **300** is ready to receive a BI **100**, cycle history, date, time, an associated IP address, etc.

The access doors **313** are configured to fit inside of the door openings **316**, and to be moved between an opened configuration (to receive or remove a BI **100**) and a closed configuration (during operation of the reader or when in stand-by). Similarly, the access door releases **314** are configured to fit inside of the door release openings **317**. As shown in FIGS. **31** and **34**, the access door releases **314** may be configured as mechanical buttons that are depressed into the door release openings **317** to actuate the access doors **313**. However, the present disclosure is not limited to such a configuration of the access door releases **314**, and indeed, any mechanism for actuating the access doors **313** can be used. In some embodiments, for example, the access door releases **314** may be electronic, and actuated by a simple touch of the access door release **314** or depression of a tactile button that triggers the relevant control board to actuate the corresponding access door **313**.

Referring to FIGS. **33-34**, each of the access doors **313** has an outer panel **313***a* that faces a user when the access door **313** is in a closed configuration, and an inner panel **313***b* that faces inside the BI reader **300** when the access door **313** is in a closed configuration. The access door **313** further includes a hook portion **313***c* at an upper portion thereof, which is connected to a pin **318** at an inner face of the front panel **311**. The hook portion **313***c* of the access door **313** is configured to pivot about the pin **318**, allowing the access door **313** to be moved between the opened configuration and the closed configuration when the access door **313** is unlocked and actuated by the access door release **314**. The front panel assembly **310** may further include a latch **320** and a latch spring **321** adjacent a lower portion of the door opening **316**. The latch **320** is configured to mate with a latch plate **313***d* at a lower portion of the inner panel **313***b* of the access door **313**. When mated, the latch plate **313***d* and the latch **320** lock the access door **313** in the closed position. And the access door release **314** is configured to release the latch plate **313***d* from the latch **320** by depressing the latch spring **321**, thereby opening the access door **313**, as discussed further below.

The access door release **314** may be located directly beneath the access door **313** (or in any other position on the front panel **311**). In some embodiments, the access door release **314** may be heat-staked onto a leaf spring, which connects the access door release **314** to the latch spring **321**. When the access door release **314** is activated (e.g., pushed

inwardly), the latch spring **321** is compressed, shifting the latch **320** and releasing the latch plate **313***d* so that the access door **313** may pivot about the pin **318** and be moved into the open configuration. According to embodiments, the front panel assembly **310** may further include one or more rotary dampers adjacent the hook portion **313***c* to dampen action of a torsion spring at the hook portion **313***c* during actuation of the access door **313**.

The access door **313** may include one or more sensors that provide signals to the control system, e.g., relating to whether the access door **313** is in the opened or closed configuration, and indicating whether the BI reader **300** is in operation. For example, the one or more sensors may include a door position sensor, which provides a signal indicating that the access door **313** is in a closed position. Responsive to a signal supplied by one or more of the sensors, the BI reader **300** (via the control system) may prohibit release of the latch plate **313***d* and lock the door **313** in place, for example, during operation of the BI reader **300**, or may prohibit the start of a detection cycle (or cycle) of the BI reader **300** if the access door **313** is in an open configuration. As another example, each of the access doors **313** may include a round segment flag **328** that passes through a slot sensor **329** as the access door **313** is opened, indicating whether the access door **313** is in an open configuration or a closed configuration.

According to embodiments, the front panel assembly **310** may further include a light source (e.g., a backlit LED) located around the periphery of the door release openings **317** such that, when lit, the light source emits a ring of light surrounding the periphery of the door release **314**. The light source may be configured to emit light in a variety of colors, for example, red, green, white, and yellow, to provide a user with an indication of the status of a cycle of the BI reader **300**. For example, in some embodiments, the light source may emit green light to indicate that the bay **375** corresponding to the access door **313** associated with the door release **314** is empty (i.e., no BI **100** is inserted), may emit red light when the bay **375** is occupied by a BI **100**, may emit white light to represent that a test is in process, and may emit a yellow light to represent a warning signal. Alternatively or additionally, the light sources of all door releases **314** may emit green light when the BI reader **300** is ready for use, and emit red light when the BI reader **300** is in operation during a detection cycle. Also alternatively or additionally, the light source of an individual door release **314** may change from red to green upon completion of a detection cycle. Also, the light source (either individually, or all of them at once) may flash red to indicate a reader fault, or may flash individually to indicate that the reader **300** detected a viable spore in the BI **100** inserted in the corresponding bay **375**. As would be understood by those of ordinary skill in the art, the light sources associated with the door releases **314** may be programmed and controlled by the control system to emit light of any color, to change from one color to another, or to flash in any of a variety of patterns to indicate various system conditions, without limitation.

As briefly discussed above, the front panel assembly **310** forms a portion of the BI reader housing **301** and provides access to the heater block assembly **370** located inside the BI reader housing **301**. Referring to FIGS. **35-36**, the heater block assembly **370** may include a first heating plate (or a lower heating plate) **371**, a second heating plate (or an upper heating plate) **372**, and a heater cartridge **373**. The second heating plate **372** is firmly mounted on the first heating plate **371** to establish a strong thermal contact between the first and second heating plates **371**, **372**. The heater cartridge **373** may be inserted into a heater channel **374** defined in the first heating plate **371**. The heater cartridge **373** may be configured to heat the first heating plate **371** to approximately 56 degrees C. to above 62 degrees C., and more preferably to approximately 60 degrees C. and may be configured to maintain a relatively constant temperature of the first heating plate **371** during operation of the BI reader **300**. For example, the heater cartridge **373** may be configured to maintain a temperature of the first heating plate **371** at a temperature of +/−2 degrees C. from a predetermined temperature (e.g., between 54 degrees C. and 64 degrees C., depending on the predetermined temperature of the heater cartridge **373**). It will be appreciated that the heater cartridge **373** is configured to heat the first heating plate **371** to a temperature that is below a maximum temperature at which the spores **181** incubate. As such, the temperature at which the first heating plate **371** is heated may differ depending on the type of spores used in the BIs **100** being tested, and thus, the temperature of the heating cartridge **373** and the first heating plate **371** is adjustable.

According to embodiments, the heater block assembly **370** is configured to reach a set temperature, e.g., 60 degrees C., within 15 minutes of operation of the heater block assembly, and to maintain (or substantially maintain) the set temperature for a prolonged period of time (e.g., during operation of the BI reader **300**).

The heater cartridge **373** is not particularly limited, and may be any suitable heating element having any size and shape so long as it is capable of fitting in a dedicated space within the first heating plate **371** and generating enough heat to maintain the first and second heating plates **371** and **372** at the selected temperature. In some embodiments, for example, the heating cartridge **373** may include a metal sheath (e.g., a 304 stainless steel sheath) having a substantially cylindrical shape and operating at 12 V/24 W that is designed for high temperature operation and to transfer heat from the heater cartridge **373** to the first and second heating plates **371**, **372**.

The first and second heating plates **371**, **372** are also not particularly limited, and may be made of any suitable material and have any size and shape so long as they are able to fit in their designated space within the BI reader **300** and maintain the selected temperature. For example, in some embodiments, the first and second heating plates **371** and **372** may be made of a metal with high thermal conductivity, e.g., an anodized metal such as aluminum, so that the first and second heating plates **371**, **372** may be efficiently heated by the heater cartridge **373**. The heater block assembly **370** may be configured to maintain a temperature that is the same (or substantially the same) across an entirety of the first heating plate **371**, such that each of the BI bays **375** (e.g., four BI bays **375**) are maintained at substantially the same temperature. As used herein, the term "substantially" is used as term of approximation, and not as a term of degree, and is intended to account for inherent deviations and inaccuracies in certain measurements, observations or properties. For example, as used herein, "substantially the same temperature" denotes that the BI bays **375** are maintained at a temperature that those of ordinary skill in the art would understand to impart no or only negligible changes in the outcome of the detection cycle associated with a particular BI bay **375**, but accounts for the possibility that not all of the BI bays **375** may be maintained at exactly the same temperature.

According to embodiments, one or more temperature sensors (e.g., thermistors) **376** may be mounted on the first heating plate **371**. The temperature sensors **376** may be

spaced apart from each other to obtain temperature readings at different locations on the first heating plate **371**. The temperature sensors **376** monitor the temperature of the first heating plate **371** and output temperature readings (e.g., with averaging) to the control system, and the control system, in response to the temperature readings may then regulate (or adjust) heat output from the heater cartridge **373** accordingly. The temperature sensors **376** may also be used to determine when the first heating plate **371** has reached the set temperature (e.g., upon start-up of the BI reader **300**), indicating that the BI reader **300** is ready for insertion of the biological indicator **100**. For example, the control system receives temperature readings from the temperature sensors **376**, and displays information regarding that reading on the display **312**. In response to the temperature readings, the control system may also activate one or more of the light sources associated with the door releases **314**. For example, upon start-up of the BI reader **300**, and upon receiving temperature readings from the temperature sensor(s) **376** that the heater block **370** (or the first heating plate **371**) has reached the threshold (or set) temperature, the control system may activate the light sources to change from red to green and/or may display a ready-for-use message on the display **312**.

One or more BI bays **375** may be formed in the first heating plate **371**. As discussed above, each of the BI bays **375** may have a shape that substantially corresponds to the obround shape of the first end **100***a* of the biological indicator **100** so that the first end **100***a* of the biological indicator **100** may be securely inserted into the BI bay **375**, e.g., with a transition fit. For example, the BI bays **375** may each have a partially obround shape, as illustrated in FIGS. **36-39**. The BI bay **375** may include a tongue **375***a* that mates with the insertion groove **138** of the BI **100** to further aid in providing proper alignment of the biological indicator **100** inside the BI bay **375**.

A lower surface of the BI bay **375** includes an opening **375***b*, which is configured to align with the imaging window **190** when the biological indicator **100** is inserted in the BI bay **375**. A BI window **379** may be located in the opening **375***b*. The BI window **379** may be transparent so that light can travel through the BI window **379** to the imaging window **190**. For example, the BI window **379** may be transparent to UV light, and in some embodiments may include a UV grade fused silica quartz, which reduces the likelihood of condensation forming on the BI window **379** during operation of the BI reader **300**. The lower surface of the BI bay **375** is configured to contact the bottom **131** of the biological indicator **100** when the biological indicator **100** is inserted into the BI reader **300**.

According to embodiments, the first heating plate **371** further includes a movable rod **380**, which contacts a movable BI presence flag **381** that is in communication with a BI presence sensor **382**. The movable rod **380** may be slidable, for example, and may be configured to partially extend into the BI bay **375** when there is no biological indicator **100** in the BI bay **375**. When the biological indicator **100** is inserted into the BI bay **375**, the biological indicator **100** moves the movable rod **380** in an insertion direction of the biological indicator **100**, which brings the movable rod **380** into contact with the movable BI presence flag **381**, thereby triggering the BI presence sensor **382**, which then communicates with the control system of the BI reader **300**.

According to embodiments, the first heating plate **371** further defines one or more BI latch openings **383** that are respectively adjacent each of the BI bays **375**. The BI latch openings **383** are configured to accommodate a BI latch **384** having a rib **387** that engages a portion of the insertion groove **138** of the biological indicator **100** (between the second end **100***b* of the biological indicator **100** and the protrusion **339**) when the biological indicator **100** is fully inserted into the BI bay **375**. The BI latch **384** is configured to lock the biological indicator **100** in place and to assist in proper alignment of the biological indicator **100** within the BI bay **375** and to reduce the likelihood of the biological indicator **100** moving after insertion into the BI bay **375**. In this way, the latch also provides additional assurance that the BI **100** is properly positioned within the BI bay **375** to align the bottom opening **132** and imaging window **190** for proper reading by the BI reader **300**, as discussed further below.

Referring to FIGS. **37-39**, according to embodiments, the BI latch **384** is movable within the BI latch opening **383** by rotating about a BI latch pin **386**. Prior to insertion of the biological indicator **100**, the rib **387** extends into the BI bay **375**, as shown in FIG. **37**. During insertion of the biological indicator **100**, the first end **100***a* of the groove **138** of the biological indicator **100** contacts the rib **387**, which helps guide insertion of the biological indicator **100** via contact between the rib **387** and the groove **138**. When the rib **387** and the insertion projection **138***a* of the BI **100** come into contact, the BI latch **384** pivots about the BI latch pin **386** and moves away from the BI bay **375** into the BI latch opening **383** to allow for insertion of the biological indicator **100**. As the biological indicator **100** is further inserted into the BI bay **375**, and when the insertion notch **138***b* of the biological indicator **100** is aligned with the rib **387**, the BI latch **384** pivots back toward the BI bay **375**, and the rib **387** is inserted into the insertion notch **138***b* of the biological indicator **100**, thereby assisting alignment of the biological indicator **100** and reducing the likelihood of the biological indicator **100** moving after insertion into the BI bay **375**. Additionally, as noted above, the alignment assistance provided by the latch imparts added assurance of the alignment of the bottom opening **132** and imaging window **190** within the BI bay **375**, as noted above. The BI reader **300** may also include a BI presence sensor, which detects the insertion of the biological indicator **100** into the BI bay **375**. The BI presence sensor may provide a signal to the control system of the BI reader **300**, to prompt the user to close the access door **313**.

The second heating plate **372** is located above the first heating plate **371**. Referring to FIGS. **40-41**, the second heating plate **372** includes one or more actuator channels **372***a* formed in an upper surface thereof, which are each configured to receive a germinant release lever **401** (see FIG. **36**). The germinant release levers **401** are configured to interact with the biological indicators **100** inserted in the respective BI bays to activate the germinant releaser **170** inside the BI **100**, as discussed further below. The second heating plate **372** further includes a plurality of upper BI bays **372***b* formed in a lower surface thereof, which correspond to the BI bays **375** formed in the first heating plate **371**.

According to embodiments, the upper surface of the second heating plate **372** may also include one or more actuator brackets (e.g., plate guides) **378** that respectively retain one or more actuators **400**. In some embodiments, for example, the second heating plate **372** may include a plurality of separate actuator bracket(s) **378**, one for each actuator **400**. However, according to some embodiments, the second heating plate **372** includes a monolithic (or otherwise connected) actuator bracket construction in which the actuator brackets **378** are connected together (or formed as a monolithic unit) to form a bracket plate that supports and

retains all of the actuators **400**. The actuators **400** may be paired with respective solenoids **405** to each activate one of the germinant release levers **401**, which interact with the BI **100** (when inserted in the respective BI bay) to actuate the germinant releaser **170**, thereby releasing the germinant **165** into the interior of the BI housing **110**. The germinant release lever **401** may include a cam surface **402** and a push rod **403**. As discussed further below, when activated, the cam surface **402** may be rotated, translating its rotation into linear movement of the push rod **403** downwardly toward the biological indicator **100**. The push rod **403** may have any suitable shape, e.g., a substantially cylindrical shape, and is configured to be inserted into the opening **121** in the first shell **120** of the BI housing **110**. As the push rod **403** moves downwardly into the opening **121**, the germinant releaser **170** is forced downward against the germinant releaser support **140**, which in turn brings the germinant releaser **170** in contact with the germinant container **160**, thereby rupturing the germinant container **160** and releasing the germinant **165** from the germinant container **160** onto the germinant pad **185**.

According to some embodiments, the actuator **400** may include a shuttle **420** (see, e.g., FIGS. **43-44**) that is configured to move linearly along a depth direction Y_R of the BI reader **300**. Each shuttle **420** may be retained by a respective actuator bracket **378** and connected to a shear wall (not shown) via a shuttle spring **410**, which is tensioned to hold the shuttle **420** in position when the BI reader **300** is not activated. According to some embodiments, each of the actuators **400** may be activated by the corresponding solenoid **405**. The solenoid **405** may activate the shuttle **420**, driving the shuttle **420** toward the front panel **311**. For example, a center rod **406** of the solenoid **405** may be driven toward the shuttle **420** along the depth direction Y_R of the BI reader **300**, overcoming the tension of the shuttle spring **410** and driving the shuttle **420** toward the front panel **311**. The shuttle **420** may include a plurality of movement bearings **423** that function as wheels, which allow for relatively easy movement of the shuttle **420**. As the shuttle **420** moves forward, a cam bearing **421** of the shuttle **420** interacts with the cam surface **402** of the germinant release lever **401**, actuating the cam surface **402** in a clockwise direction. A wave spring **424** may surround the cam bearing **421**, which applies contact pressure on the cam surface **402** as the cam bearing **421** rides over the cam surface **402**. The push rod **403** then extends downwardly toward the BI bay **375** (and into the opening **121** in the BI housing **110**). After completion of a test cycle, the solenoid **405** retracts the center rod **406**, and the shuttle **420** is returned to its starting position by the shuttle spring **410**, disengaging the germinant release lever **401** from the biological indicator **100**. The solenoid **405** is not particularly limited, and may be any suitable solenoid capable of actuating the shuttle **420** as described herein. In some embodiments, for example, the solenoid **405** may be a push tubular solenoid, for example, a 1" dia.×2" push solenoid.

The BI reader **300** may include one or more sensors that monitor the location of the shuttle **420**, such as a solenoid forward limit sensor, which senses whether the solenoid **405** is activated and the shuttle **420** is advanced (e.g., the center rod **406** is driven to the shuttle **420**) and a solenoid return limit sensor, which senses whether the solenoid **405** is deactivated and the shuttle **420** is retreated (e.g., the center rod **406** is retracted). The solenoid forward limit sensor and the solenoid return limit sensor may provide a signal to the control system of the BI reader **300**, to assist in determining whether the access door **313** of the BI bay **375** is locked or if the BI bay **375** is accessible.

The shuttle **420** may include a door interlock spring **422**, which is configured to engage with a retaining clip **319** adjacent the pin **318** of the access door **313**, as illustrated in FIG. **45**. For example, the door interlock spring **422** may interact with the retaining clip **319** to prevent rotation of the access door **313** while the shuttle **420** is advanced toward the front panel **311**. When the shuttle **420** is retracted toward the rear panel **391**, the door interlock spring **421** moves away from the retaining clip **319**, thereby unlocking the access door **313** at the hook portion **313***c*. The door interlock spring **422** provides an additional locking mechanism that prevents movement of the access door **313** during a test cycle of the BI reader **300**.

The second heating plate **372** may further include a lever return spring **385** (see FIG. **42**), which is tensioned to drive the germinant release lever **401** back to a starting position (and to move the push rod **403** up and out of the opening **121**) when the actuator **400** is retracted.

The shuttle **420** may further include one or more shuttle flags **425** and/or corresponding sensors, which are used to communicate a location of the shuttle **420** to the control system of the BI reader **300**. As such, the control system of the BI reader **300** may receive a signal from the shuttle flag/sensor **425** that the shuttle **420** has moved, indicating that the designated BI bay **375** has been actuated, which the control system may then use to signal that the BI bay **375** is active and/or to activate the optical assembly.

It will be appreciated that although the actuator **400** is described herein in connection with the shuttle **420**, any suitable actuator or actuation mechanism that allows for activation of the germinant releaser **170** when the BI **100** is inserted in the BI bay **375** may be used, and the present disclosure is not limited to the specifically described actuator embodiments.

According to embodiments, the control system may include a lower BI sensor board **389** (shown in FIG. **36**), which may be located above the actuator bracket(s) **378**. The lower BI sensor board **389** may include sensors that are configured to detect the presence (or absence) of the biological indicator **100** in the BI bays **375** and/or to detect a location of the actuators **400**. The lower BI sensor board **389** may be spaced apart from the second heating plate **372** via the actuator bracket(s) **378**, thereby reducing the likelihood of damage to the lower BI sensor board **389** while the second heating plate **372** is heated (or held at an elevated temperature).

The heater block assembly **370** serves to heat the biological indicator **100** when it is inserted in the corresponding BI bay **375** to allow for germination of the spores **181**. The heater block assembly **370** also provides datum locations for the biological indicator **100** for illumination and imaging of spore imaging areas inside the biological indicator **100**. The heater block assembly **370** may include a self-calibration target **369** at a lower surface of the first heating plate **371**, which allows for calibration of the positioning assembly **340** (discussed further below) and the heater block assembly **370**. According to some embodiments, the self-calibration target **369** may include a substrate (e.g., soda lime glass) having a substantially square shape and offset, angled parallel striping, which may be utilized to calibrate the positioning assembly **340** during operation.

As shown in FIG. **47**, the heater block assembly **370** is located in an upper portion of the BI reader housing **301** (e.g., along a height direction Z_R of the BI reader **300**), and the positioning assembly **340** is located in a lower portion of

the BI reader housing **301**. However, the present disclosure is not limited to this configuration, and any configuration of the subassemblies of the BI reader **300** (including the heater block assembly **370** and positioning assembly **340**) may be used so long as the BI reader can function as described herein.

Referring to FIGS. **48** and **49**, the positioning assembly **340** includes a stepper motor **341** and belt drive **342** which move a scan head assembly **350** below the BI bays **375**. The stepper motor **341** may drive the belt drive **342**. The stepper motor **341** is not particularly limited, and may include any such motor capable of driving the belt drive **342**. In some embodiments, for example, the stepper motor **341** may include a high torque motor with an integrated brake system, which is mounted on a deck **345** with a linear guide block **343** riding in a guide rail **343***a* adjacent thereto in a width direction X_R of the BI reader **300**. The belt drive **342** is also not particularly limited, and may have any suitable construction. In some embodiments, for example, the belt drive **342** may include a drive pulley **342***a*, an idler pulley **342***b*, and a timing belt **342***c*. The timing belt **342***c* and the linear guide block **343** may extend parallel to each other along the width direction X_R, such that as the belt drive **342** is driven by the stepper motor **341**, the linear guide block **343** moves along the width direction X_R. According to some embodiments, the positioning assembly **340** may be configured to move a load at 60 mm per full revolution, however, the present disclosure is not limited thereto. According to embodiments, the stepper motor **341** may include a magnetic brake (e.g., an integrated magnetic brake), which prevents (or reduces the likelihood of) movement of the linear guide block **350** (on which the scan head assembly **350** is situated) when the BI reader **300** is not in use. According to embodiments, the timing belt **342***c* may be a circular tooth profile GT belt, but the present disclosure is not limited thereto, and the timing belt **342***c* may have any suitable construction. In use, the stepper motor **341** drives the driver pulley **342***a* causing it to rotate, which in turn causes the timing belt **342***c* to rotate around the idler pulley **342***b* and the linear guide block to translate linearly along the guide rail **343***a*.

According to some embodiments, the positioning assembly **340** may further include one or more threshold sensors to limit the movement of the scan head assembly **350** past one or more threshold limits. For example, in some embodiments, the positioning assembly **340** may include one sensor to the right of the scan head assembly **340**, and another sensor to the left of the scan head assembly **340** to thereby limit movement of the scan head assembly **340** in both directions along the belt drive **342**.

The scan head assembly **350** is mounted on the linear guide block **343**. Referring to FIG. **49**, the scan head assembly **350** includes an excitation source (e.g., an ultraviolet light emitting diode (UV LED) excitation source) **351**, an emission lens (or an excitation focus lens) **352**, a collection lens **353**, an excitation filter **354**, and a first mirror **355**. The emission lens **352** and the collection lens **353** may be bonded (e.g., permanently bonded) in place using an adhesive (e.g., a UV curable adhesive) or any other suitable bonding means. The excitation source **351** is attached to a bracket **356**, which is fastened to a scan head body **357**, e.g., via screws. As such, the excitation source **351** may be actively aligned with the scan head assembly **350**. According to embodiments, the first mirror **355** may be pressed to a datum using springs (e.g., urethane tubing springs). The scan head assembly **350** may further include a scan head temperature sensor **358** (e.g., a thermistor) at the scan head body **357**, which monitors the temperature of the scan head assembly **350**.

The excitation source **351** may be configured to emit light in the UV light wavelength range, i.e., in a wavelength range of about 100 to about 400 nm. In some embodiments, for example, the excitation source **351** may be configured to emit light in a range of about 200 to about 300 nm, or about 250 to about 300 nm. For example, in some embodiments, the excitation source **351** may have a peak wavelength of between about 270 nm and about 285 nm. The excitation filter **354** may have a center wavelength of between about 270 nm and about 370 nm, and for example may have a center wavelength of about 330 nm, and may be placed between the excitation source **351** and the imaging window **190** of the bioindicator **100**. Light emitted from the excitation source **351** passes through the emission lens **352** and the excitation filter **354** of the scan head assembly **350** and through the imaging window **190** of the BI **100** to the spores **181** on the spore carrier **180** inside the biological indicator **100**. Light emitted by the spores **181** is then emitted downwardly, back through the imaging window **190**, the BI window **379** in the heater block assembly **370**, the collection lens **353**, and to the first mirror **355**, which reflects the light along the width direction X_R to a second mirror (e.g., a turning mirror) **331**, which then reflects the light along the depth direction Y_R to the camera assembly **360**, which captures an image of the light.

More specifically, when the BI **100** is inserted into the reader, and the germinant **165** is released inside the BI **100**, the photoluminescent component (e.g., Tb ions) may bind to any DPA released from the spores that were killed during the sterilization cycle. Additionally, any spores that were not killed by the sterilization process will begin to germinate on contact with the germinant component (e.g., L-alanine) of the germinant solution, which germination will cause those spores to also release DPA, which will in turn bind to the photoluminescent component and begin to luminescence in response to the light from the excitation source. When the spores (or more accurately, the DPA-photoluminescent complex) begin to luminesce, that luminescence is emitted back through the imaging window **190** of the BI along the optical path described above to the camera assembly **360**, which captures images of the luminescence. The BI reader **300** analyzes the images captured by the camera assembly **360** to determine whether any of the spores **181** survived the sterilization cycle, as discussed further below. In particular, in some embodiments, the BI reader **300** detects a static background level of DPA from the luminesce returned by spores that were killed during the sterilization process. If any spores were not killed during the sterilization process, they will germinate upon contact with the germinant solution **165**, and will release DPA upon germination, which the BI reader **300** will detect as a DPA signal above the static background level (when present). And the BI reader **300** will associate any DPA signal above the static background level, or any DPA signal occurring after a predetermined period of time after BI activation, with failure of the sterilization process.

The emission lens **352** may be located between the excitation source **351** and the excitation filter **354** to disperse the light emitted from the excitation source **351**. According to embodiments, the emission lens **352** may be a double-convex lens having a UV-AR coating. According to embodiments, the emission lens **352** may include a fused silica with a design wavelength of between approximately 250 nm and approximately 425 nm. According to embodiments, the

emission lens **352** may have a 12 mm diameter, a 12 mm focal length, and a 9¼ mm back focal length.

According to one or more embodiments, the scan head assembly **350** is mounted on the linear guide block **343**, which moves along the guide rail **343***a* which is aligned beneath the BI bays **375**. The first mirror **355** is located on the bracket **357**, and is oriented (or aligned) such that the first mirror **355** reflects light along the width direction X_R to the second mirror **331** on a mirror mount **330** (see, e.g., FIG. **47** and FIG. **50**), thereby relaying a collimated emission ray from the scan head assembly **350** to the camera assembly **360**. The camera assembly **360** is attached to a bottom plate **302** of the BI reader housing **301**, e.g., via mounting brackets. The camera assembly **360** is located in a pocket edge of the bottom plate **302**. While the scan head assembly **350**, camera assembly **360**, and optical path are described above with reference to particular locations and directional light paths, it is understood that these components can be alternately positioned or located so long as the resulting optical path is capable of delivering light from the scan head assembly **350** to the spore carrier **180**, and returning the luminescence from the spore carrier to the camera assembly **360**.

Referring to FIG. **47**, in some embodiments, the linear guide block **343** is separated from the mirror mount **330** by a central panel **304** that extends along the width direction X_R. The central panel **304** may define a first opening **304***a* aligned with the first mirror **355** and the second mirror **331**, which allows light to be reflected from the first mirror **355** to the second mirror **331**. The central panel **304** may also define a second opening **304***b* to accommodate the timing belt **342***c*.

In some embodiments, the mirror mount **330** is stationery and may be located adjacent to the belt drive **342**. The mirror mount **330** may be mounted on the deck **345** between the stepper motor **341** and the scan head assembly **350**, for example, between the stepper motor **341** and the central panel **304**. According to embodiments, the mirror mount **330** may be aligned with the scan head assembly **350** along the width direction X_R and aligned with the camera assembly **360** along the depth direction Y_R, and is therefore configured to reflect light from the scan head assembly **350** to the camera assembly **360**.

The mirror mount **330** may have any suitable configuration such that the mirror mount **330** may receive the second mirror (turning mirror) **331** and reflect light from the scan head assembly **350** to the camera assembly **360**. For example, referring to FIG. **50**, the mirror mount **330** may include a base portion **332** and a bracket portion **333**. The base portion may have any suitable height such that the second mirror **331** is properly aligned with the scan head assembly **350** along the height direction Z_R to deliver light to the camera assembly **360**. The bracket portion **333** is configured to receive and hold the second mirror **331**, and may have a pair of connecting side walls **334**, a generally triangular shaped upper wall **336**, and a base **335** on which the second mirror **331** sits. The side walls **334** each have an opening **334***a* that allows light to pass therethrough and onto the second mirror **331**. The second mirror **331** may have a triangular prism shape (e.g., a right angle mirror) and may include a silver coated N-BK7 substrate, though the present disclosure is not limited thereto, and the second mirror may have any suitable shape and construction.

Referring to FIGS. **51**A, **51**B, **52**A, and **52**B, the camera assembly **360** may include a camera **361**, an optical lens **362**, a filter **363**, and a camera fan **364** and Peltier assembly **365** (for keeping the camera at safe operating temperatures). According to embodiments, the camera assembly **360** may be located in a fixed position relative to the BI housing **301**, and at the end of the optical path described above for receiving the luminescence from the spores. This configuration (i.e., a moving scan head assembly and a fixed camera assembly) enables use of only one camera **361** to analyze multiple bays. However, the present disclosure is not limited to this configuration, and the BI reader **300** may instead include a camera **361** for each BI bay **375**. In such embodiments, the BI reader **301** may also include a scan head assembly **350** for each BI bay, and both the scan head assemblies **350** and the cameras **361** may be fixed in position beneath their respective BI bay **375**. As will be appreciated, such a multiple-camera, multiple-scan head construction would eliminate the need for the positioning assembly **340** and simplify the optical path from the imaging window **190** of the BI to the camera (as the turning optics (i.e., the first and second mirrors and the mirror mount) would no longer be necessary), but would significantly increase the cost and size of the reader.

According to example embodiments, the camera **361** may be a thermoelectrically (TE)-cooled charge-coupled device (CCD) camera. For example, in some embodiments, the camera **361** may be a high-power camera, meaning that it allows for an imaging rate (or frame frequency) of about 5 kHz to about 10 kHz, which allows for effective imaging of the lifetime of the fluorescence signal of the spores **181**. The camera **361** may be configured to operate in a time-gated mode for capturing long living luminescence of the spores **181** when excited with UV (e.g., UVC) radiation by flashing UV light and exposing the camera **361** using electronic shutter at regular intervals. The camera **361** may also be configured to operate in a bright image mode for a variable exposure at a frequency of between about 1 ms to 2000 ms. The optical lens **362** is connected to the camera **361**. The optical lens **362** may, for example, have a focal length (FL) of 35 mm and a minimum working distance of 165 mm (f/1.65) (i.e., a minimum working distance of 165 mm or greater). The filter **363** is connected to the lens **362**. The filter **363** may be a band pass filter, for example a filter between about 534 nm to about 566 nm. In some embodiments, the filter **363** may be a 550 nm band pass filter.

Referring to FIGS. **52**A-**52**B, the Peltier assembly **365** may be mounted to the camera **361**, and the fan **364** may be mounted to the Peltier assembly **365** to cool the camera **361**. According to embodiments of the present disclosure, a camera guard **366** having a plurality of openings **367** may be attached to the fan **364** to reduce the likelihood of any foreign objects entering the fan **364** and the Peltier assembly **365**. The Peltier assembly **365** may be utilized to improve performance of the fan **364**, e.g., to improve heat transfer characteristics while the fan **364** cools the camera **361**. According to embodiments, the fan **364** may include a 40×40×20 24 VDC VAPO® 7.7 CFM fan (VAPO® is a registered trademark of Sunonwealth Electric Machine Industry, Co.). The guard **366** may be attached to the fan **364**, and may be made of a durable material, such as a metal. For example, the guard **366** may include an aluminum alloy. The openings **367** may be formed radially, for example, the guard **366** may include 12 of the openings **367**, with symmetrical rounded wedge shapes. However, it is understood that the guard **366** is not limited thereto, and can have any suitable configuration and any suitable number and shape of the openings **367**.

Referring to FIGS. **53-54**, the BI reader housing **301** further includes an upper housing panel **306** at a top thereof and a lower housing panel **307** below the bottom plate **302**

and at a bottom of the BI reader **300**. The upper housing panel **306** and the lower housing panel **307** may each have a substantially U-shaped profile such that the upper housing panel **306** and the lower housing panel **307** extend along the height direction Z_R of the BI reader **300** and mate with each other, forming the sides of the BI reader housing **301**.

The rear panel assembly **390** includes a rear panel **391**, one or more axial fans **392**, and an air intake plenum **393**. As illustrated in FIG. **53**, the rear panel **391** may have a plurality of perforations **394** that permit air flow therethrough. The shape and number of the perforations **394** is not particularly limited, and may be any shape and number so long as the perforations **394** allow a sufficient amount of air flow through the rear panel **391**. For example, in some embodiments, as shown in FIG. **53**, the perforations **394** may take the shape of vertical slots such that the rear panel **391** resembles a grate. The axial fans **392** and the air intake plenum **393** each allow for the intake of air into the BI reader **300**, which may then exit through vents below the front panel **311**. For example, ambient air may be drawn from an area behind the BI reader **300** into the BI reader **300** through the rear panel assembly **390**. Positive pressure is then built inside the BI reader **300**, which expels warm air through the vents at the front panel assembly **310**. As an example, one of the axial fans **392** may be located directly adjacent the camera **361**, and two other axial fans **392** may be located near the heater block assembly **370** and provide additional air flow. As such, the amount of dust and other particulates in the system may be reduced. The axial fans **392** may be used to maintain a suitable temperature of the BI reader **300** for the components contained therein, for example, to keep the camera **361** at a suitable operating temperature while being used in close proximity to the heater block assembly **370**.

Turning back to FIG. **47**, according to embodiments, the heater block assembly **370** is located above the linear guide block **434**. As discussed above, the central panel **304** defines the second opening **304***b* that accommodates the timing belt **343**. The stepper motor **341** and the drive pulley **342***a* may be located between a first side **303***a* of the BI reader housing **301** and the central panel **304**, and the idler pulley **342***b* and the linear guide block **343** (as well as the scan head assembly **350** mounted on the linear guide block **343**) may be located between a second side **303***b* of the BI reader housing **301** and the central panel **304**. The heater block assembly **370** may be supported between the second side **303***b* and the central panel **304** so that it is located on the same side of the BI reader **300** as the linear guide block **343**. The camera assembly **360** and the mirror mount **330** are both located between the first side **303***a* and the central panel **304**.

According to embodiments, the BI reader **300** includes four access doors **313** which respectively correspond to four BI bays **375** spaced apart from each other along the width direction W_R of the BI reader **100**. As such, the BI reader **300** can perform sterilization efficacy testing on four biological indicators **100** concurrently (or simultaneously) during one detection cycle of the BI reader **300**.

FIG. **55** depicts a block diagram of a control system according to embodiments of the present disclosure. According to some embodiments, the control system **500** may be configured to operate the positioning assembly **340**, the heater block assembly **370**, the access doors **313** and solenoids **405**, the camera assembly **360**, the excitation source **351** and scan head assembly **350**, the display **312**, etc. of the BI reader **300**. In some embodiments, the control system **500** may include a plurality of microcontrollers (or processors) that run one or more modules configured to control different aspects of the BI reader **300**. For example, the one or more processors of the control system **500** may run a positioning assembly control module **510**, a BI bay heater control module **520**, a BI bay door and handler control module **530**, a camera control module **540**, an excitation control module **550**, and a user interface control module **560**. For example, in some embodiments, the one or more controllers of the control system **500** may include a control processor **501**, a bay processor **503** and a display processor **502**, each of which may operate one or more of the positioning assembly control module **510**, BI bay heater control module **520**, BI bay door and handler control module **530**, camera control module **540**, excitation control module **550**, and user interface control module **560**.

In some embodiments, as shown generally in FIGS. **55** and **56**, the positioning assembly control module **510** may be configured to control the positioning assembly **340**. For example, the positioning assembly control module **510** may run the stepper motor **341** and the belt drive **342**. Additionally, in some embodiments, the positioning assembly control module **510** may include lock-out logic to prevent the positioning assembly **340** from advancing the scan head assembly **350** past a preset threshold limit (as discussed further below in connection with the bay processor, and above in connection with the positioning assembly **340**). In some embodiments, the positioning assembly control module **510** may be run by the control processor **501**, as discussed more below.

As shown in FIGS. **55** and **57**, the BI bay heater control module **520**, according to some embodiments, may be configured to control the heater cartridge **373** of the heater block assembly **370** and the axial fans **392**, and receive and process signals from the temperature sensors **376** of the heater block assembly **370**. The BI bay heater control module **520** may further include logic to inhibit continued operation of the heater cartridge **373** if the temperature sensor(s) **376** register a temperature difference above a preset threshold. The BI bay heater control module **520** may further run a heater current monitor, and include logic to inhibit continued operation of the heater if the heater current monitor registers a current exceeding a preset threshold. In some embodiments, the BI bay heater control module **520** may be run by the bay processor **503**, as discussed more below.

In some embodiments, as shown in FIGS. **55** and **58**, the BI bay door and handler control module **530** may be configured to control the solenoids **405**. This module may further communicate with one or more sensors within each BI bay for detecting various conditions. In some embodiments, the BI bay door and handler control module **530** may communicate with these sensors via one or more BI sensor boards (e.g., an upper BI sensor board **506** and lower BI sensor board **505**). For example, in some embodiments, the BI bay door and handler control module **530** may communicate with one or more of a door position sensor, a solenoid forward limit sensor, a solenoid return limit sensor, or a BI presence sensor. Each of these sensors may be an infrared photo-interrupter, as discussed above, and each of the BI bays may include one, any combination of two or more, or all of these sensors. In some embodiments, the BI bay door and handler module **530** may be run by the bay processor **503**.

As shown in FIGS. **55** and **59**, the camera control module **540**, according to embodiments, may be configured to control the camera **361**. For example, the camera control module **540** may be configured to operate the camera, and receive and process the images received by the camera **361**.

In some embodiments, the camera control module **540** may be run by the control processor **501**.

According to some embodiments, as shown in FIGS. **55** and **60**, the excitation control module **550** may be configured to operate the excitation source **351**. In some embodiments, the excitation control module **550** may be configured to receive input from the BI bay door and handler module **530** regarding, for example, signals indicative of which of the BI bays **375** are occupied by a BI **100**. The excitation control module **550** may process that input to determine which of the BI bays **375** require excitation source turn-on, and which of the BI bays **375** can be skipped in a particular run (e.g., because a particular BI bay **375** does not have a biological indicator **100** inserted therein). The excitation control module **550** may also operate a built-in mechanism to regulate the current of the excitation source **351** to maintain current regulation through cycles (e.g., PWM cycles) of the excitation source **351**. The excitation control module **550** may also be configured to control the timing of excitation source turn-on and its length of exposure, and the timing of camera turn-on and its length of exposure. In some embodiments, aspects of the excitation control module **550** may be run by the control processor **501**, and other aspects of the excitation control module **550** may be run by the bay processor **503**. However, the present disclosure is not limited thereto, and it is understood that the excitation control module **550** may be run by a single processor (e.g., either the control processor **501** or the bay processor **503**).

As shown in FIGS. **55** and **61**, the user interface control module **560**, in some embodiments, may be configured to manage interaction of the user with the display **312** (e.g., the touch panel). For example, the user interface control module **560** may be configured to receive and process user input, and manage display of information to the user on the display **312**. In some embodiments, the user interface control module **560** may be run by the display processor **502**.

As noted above, to accomplish control of each of these modules, the control system may include a plurality of microcontrollers (or processors). For example, in some embodiments, the control system may include at least a control processor **501**, a display processor **502**, and a bay processor **503**.

In some embodiments, for example, the control processor **501** may be configured to run at least portions of the positioning assembly control module **510**, the camera control module **540**, and the excitation control module **550**. Running one or more of these modules, the control processor **501** may be utilized for system supervision, managing the camera **361** and the positioning assembly **340** (or more specifically the stepper motor **341**), operating the camera **361** and the excitation source **351**, processing and receiving images captured by the camera **361**, sequencing spore detection tests, and managing the light sources at the door openings **316** (also referred to as a front panel LED board **504**). To manage the light sources at the door openings **316**, the control processor **501** may be configured to communicate with a front panel LED (or light source) board which includes the light source circuitry.

Additionally, to control the positioning assembly **340**, in some embodiments, the control processor **501** may include lock-out logic to prevent the positioning assembly **340** from advancing the scan head assembly **350** past a preset threshold limit. In such embodiments, the positioning assembly **340** may further include one or more threshold sensors (as discussed generally above) to limit the movement of the scan head assembly **350** past one or more threshold limits. For example, in some embodiments, the positioning assembly **340** may include one sensor to the right of the scan head assembly **340**, and another sensor to the left of the scan head assembly **340** to thereby limit movement of the scan head assembly **340** in both directions along the belt drive **342**.

In some embodiments, the BI reader **300** may include an external USB diagnostic port (not shown) and/or an Ethernet port (also not shown). In embodiments including the USB diagnostic port, the control processor **501** may support the USB diagnostic port, and host a diagnostic graphical user interface (GUI). And in embodiments including the Ethernet port, the control processor **501** may be configured to facilitate the exchange of BI test data with Instrument Tracking Systems (e.g., within the hospital) to comply with data management requirements.

Additionally, in some embodiments, the display processor **502** may run the user interface control module **560**. Running this module, the display processor may be configured to manage the display **312**, including the touch panel (when used), and to receive and process user inputs. The display processor **502** may also support an ethernet connection.

The bay processor **503**, according to some embodiments, may be configured to run at least portions of the BI bay heater control module **520**, and the BI door and handler module **530**. Running these modules (or portions thereof), the bay processor **503** may be configured to operate the solenoids **405**, monitor and report statuses (or configurations) of the access doors **313**, operate the heater cartridge **373**, operate the axial fans **392**, and manage certain functions of the excitation source **351**. As shown in FIGS. **55** and **58**, the bay processor **503** may also be configured to communicate with an upper BI sensor board **506** and the lower BI sensor board **389** which include the circuitry for the various BI sensors, including, for example, the slot sensors **329**, the BI presence sensors **382**, and the shuttle sensors **425**. As shown in FIGS. **55** and **57**, the bay processor **503** may also be configured to communicate with the temperature sensors **376** of the heater block assembly **370**, and process signals from those sensors to control operation of the heater cartridge **373** and axial fans **392** in order to maintain the temperature of the heater block assembly **370** within the temperature range discussed above.

It will be appreciated that the heater block assembly **370** and the optical assembly (i.e., the positioning assembly **340** and the camera assembly **360**) are calibrated with each other to provide parallelism between each of the BI bays **375** and the scan head assembly **350**, such that a distance between the scan head assembly and each of the BI bays **375** is constant and such that the scan head assembly captures images at a focal plane for each of the BI bays **375**. It will further be appreciated that other configurations are possible. For example, the camera assembly **360** could be located in a different portion of the BI reader housing **301** and the mirror mount **330** moved or omitted, provided that the camera assembly **360** is located such that it can receive light transmitted by the BI **100** with minimal (or reduced) interference. As another example, separate camera assemblies **360** and/or separate excitation sources **351** could be utilized for each BI bay **375**, as described above. However, the present disclosure also provides for a BI reader **300** in a compact housing **301**, which allows for the use of fewer components and analysis of multiple BI bays **375** without the use of separate excitation and reading equipment for each BI bay **375**, thereby reducing the size and cost of the reader, as also discussed above.

According to embodiments of the present disclosure, a method of detecting the sterilization efficacy of a sterilization run includes utilizing the BI reader **300** and at least the

BI **100** (and in some embodiments, the PCD **200**) discussed above. According to embodiments, for example, the BI reader **300** may be utilized to test and analyze the biological indicator **100** in order to determine whether a sterility procedure to which the biological indicator **100** was exposed was successful.

First, the user may activate the BI reader **300**, for example, by pressing an on/off button or interacting with the display **312** in the front panel **311** of the BI reader **300** (e.g., to wake the BI reader **300**). Upon receiving such user input, the control processor activates the heater cartridge **373** to begin warming the heater block assembly **370**, e.g., the first heating plate **371** and the second heating plate **372**. When the first heating plate **371** and the second heating plate **372** are brought to a sufficient temperature, e.g., 60 degrees Celsius, the temperature sensor(s) **376** on the heater block assembly **370** send a signal to the control processor, and the control processor provides an indication to the user that BI reader **300** is ready for use. The indication may be via information displayed on the display **312**, and/or may be via a change in the light sources associated with the access door releases **314**. For example, the change in the light sources may be a change from off to on (or vice versa), a change in color (such as from red to green), or a change from on (or off) to flashing.

To perform the sterilization efficacy test, the user may depress (or otherwise actuate) the access door release **314**, thereby releasing the access door **313** and exposing the door opening **313** and the chamber **326**. The user may then insert the biological indicator **100** into the door opening **313**, through the chamber **326** and the chamber opening **327**, thereby inserting the first end **100***a* of the biological indicator **100** into the BI bay **375**. As the first end **100***a* of the biological indicator **100** is inserted into the BI bay **375**, the chamber **326** guides the biological indicator **100** to the chamber opening **327** and the BI bay **375**, as discussed above. As the first end **100***a* of the biological indicator **100** continues to be moved inside the BI bay **375**, the insertion groove **138** contacts the BI latch **384**, which then pivots about the BI latch pin **386** and into the BI latch opening **383** to allow for proper insertion of the biological indicator **100**. As the biological indicator **100** is being inserted into the BI bay **375**, the BI latch **384** (e.g., the rib **387**) moves toward the biological indicator **100** by means of the insertion notch **138***b*, and the rib **387** moves into the insertion notch **138***b* to hold the biological indicator **100** in place.

One biological indicator **100** may be inserted into each BI bay **375**. As such, according to embodiments, for a BI reader **300** having four BI bays **375**, four biological indicators **100** can be tested concurrently or simultaneously. However, it is not necessary for all of the BI bays **375** of the BI reader **300** to be occupied in order to run a detection cycle. Rather, any number of the BI bays **375** may remain empty such that a detection cycle can be run on only a single BI **100** (with all remaining bays empty), or any other number of BIs (up to the total number of bays on the reader). In such a case, the control system of the BI reader **300** receives a signal from the BI presence flag or sensor associated with each BI bay **375**, and directs the scan head assembly **350** to only scan (or test) those BI bays **375** that are occupied by a BI **100**. As a result, during the detection cycle, the scan head assembly **350** will move from bay to bay, but will only emit light from the excitation source into the BI bays **375** that are occupied. While the scan head assembly **350** may stop below the empty bays, the excitation source will not be activated at the empty bays **375**. Alternatively, the control system may direct the scan head assembly **350** skip the empty bays altogether, so that the scan head assembly **350** does not stop at the empty bays, and moves only between the bays that are occupied.

After the biological indicator **100** is inserted into the BI bay **375**, the user may close the access door **313**, e.g., by actuating the access door release **314** again, or by manually lowering the access door. After all access doors **313** are closed, the BI reader **300** may perform a variety of software checks to ensure the BI reader **300** is ready to perform the test. For example, utilizing the scan head assembly **350** and/or the camera assembly **360**, the control system may initiate a dust check to check for dust particles in the optical path by checking for high frequency noise in the field of view of the scan head assembly (e.g., the field of view defined by the BI window **379** of the bay **375**), indicating the presence of foreign matter in the optical path (e.g., between the BI window **379** of the BI bay **375** and the imaging window **190** of the BI **100**). The BI reader **300** may also conduct a condensation check to check for condensation formed on the BI window **379** during heating of the heater block assembly **370**. The BI reader **300** may also perform an alignment check of the biological indicator **100** to ensure that the BI window **379** is properly aligned in the BI bay **375**, for example, by detecting the Odin's cross shape of the bottom opening **132** and confirming that the biological indicator **100** has been inserted within acceptable tolerances. The BI reader **300** may also perform a positioning check to ensure proper calibration of the scan head assembly **350** and the positioning assembly **340** and a correct distance between the scan head assembly **350** and the heater block assembly **370** (and therefore the BI window **379**). The self-calibration target **369** may be utilized to check for proper calibration of the scan head assembly **350** and the positioning assembly **340** by emitting light toward the self-calibration target **369** and measuring a pattern reflected from the calibration target **369** to ensure proper distancing between the scan head assembly and the heater block assembly **370**. If any of these systems checks fail, the control processor will deliver a fault or error message, which may include fault or error information displayed on the display **312**, and/or may be via a change in the light sources associated with the access door releases **314**. In addition, the BI reader **300** may include a z-focus adjustment via the optical assembly to estimate any deviation from the ideal focal plane (e.g., range finding) of the spore carrier **180** during a test cycle. The z-focus adjustment may be accomplished by utilizing an electronically controlled micrometer with the scan head assembly **350** such that a focal distance of the collection lens **353** may be adjusted within a range of +/−250 μm.

If the systems checks all pass, the control system (via, e.g., the control processor) activates the solenoid **405** to push the center rod **406** of the solenoid **405** toward the shuttle **420** along the depth direction Y_R of the BI reader **300**, thereby overcoming the tension of the shuttle spring **410** and driving the shuttle **420** toward the front panel **311**. The activation of the solenoid **405** effectuates locking of the access doors **313** in the closed position. As the shuttle **420** moves forward, the cam bearing **421** of the shuttle **420** interacts with the cam surface **402** of the germinant release lever **401**, actuating the cam surface **402** in a clockwise direction. The push rod **403** then extends downwardly toward the BI bay **375** and into the opening **121** in the BI housing **110**. Additionally, the door interlock spring **422** of the shuttle **420** engages with the retaining clip **319** to lock and prevent rotation of the access door **313** while the shuttle **420** is activated.

The push rod **403** extends downwardly through the opening **121** of the BI housing **110**, applying pressure on the

germinant releaser **170**, which in turn, applies pressure on the germinant releaser support **140**, which together with the germinant releaser **170** applies pressure against the germinant container **160**, thereby rupturing the germinant container **160** and releasing the germinant **165** contained therein into the interior of the BI **100**. The germinant **165** saturates the germinant pad **185** which wicks the germinant through the germinant pad **185** onto the spore carrier **180** which contains the spores **181** on an underside thereof. The germinant **165** then wicks through the spore carrier **180** to reach the spores on the underside thereof.

As discussed above, when the spores **181** on the spore carrier **180** are killed during the sterilization run, those spores release DPA. When those spores (or more accurately, the DPA released from those spores) come in contact with the germinant solution **165**, the photoluminescent component of the germinant solution (e.g., Tb ions) may bind to the DPA to form a photoluminescent complex (e.g., a Tb-DPA complex) that will luminesce upon activation by UV light. After the germinant **165** is released inside the biological indicator **100**, the control system may activate the optical assembly, which generates, captures, and analyzes images of the activity inside each biological indicator **100**. More specifically, the control system activates the positioning assembly to move the linear guide block **343** along the guide rail **343***a* to align the scan head assembly **350** beneath the first occupied BI bay **375**. The scan head assembly **350** then emits light from the excitation source **351** toward the BI window **379**, which light passes through the emission lens **352**, the excitation filter **354**, the BI window **379**, and the imaging window **190** to the spores **181** inside the biological indicator **100**. This activates the photoluminescent complex, which begins to luminescence and emit back toward the imaging window, along the optical path described above (i.e., through the imaging window **190**, the BI window **379** in the heater block assembly **370**, the collection lens **353**, to the first mirror **355**, which reflects the light along the width direction X_R to the second mirror **331**, which then reflects the light along the depth direction Y_R to the camera assembly **360**) to the camera **361**. In some embodiments, the camera **361** captures the luminescence generated by the dead spores as a bright, static background image. However, it is understood that in some embodiments, the camera may not capture a background image.

As also discussed above, when any spores **181** on the spore carrier **180** survive the sterilization cycle, these viable (or live) spores will begin to germinate upon contact with the germinant (e.g., L-alanine) in the germinant solution **165**. Upon germination, these live spores will release DPA, which may then bind to the photoluminescent component of the germinant solution **165**. The resulting DPA-photoluminescent complex will then luminesce upon activation with UV light, as described above in connection with the dead spores. However, because the live spores release their DPA after germination, there is a time-lapse and an amplitude increase between any DPA signal received by the camera from the dead spores, and the DPA signal received by the camera from the live spores. Accordingly, when the camera detects a DPA signal that is above the static background signal from the dead spores, the control system returns an indication that the sterilization cycle failed. This indication can be via information displayed on the display **312**, and/or via a change in the light sources associated with the access door releases **314** and/or via an audio alarm.

Prior to running the detection protocols, the control system may also run a check using the optical assembly to initially detect whether the germinant **165** was successfully released, thereby saturating the spore carrier **180**. The optical assembly and control system conduct this check by detecting and calculating the average intensity of light emitted over time. For example, if the control system and optical assembly detect an intensity change at or above a specified threshold intensity ratio (e.g., approximately 110%) over time, the BI reader **300** registers the germinant **165** as having been successfully released, and proceeds with the detection cycle. However, if the control system and optical assembly detect an intensity that is lower than the specified threshold intensity, the BI reader **300** registers the germinant as not having been adequately released, and returns a fault or error. As discussed above, the fault or error may be indicated via information displayed on the display **312**, and/or may be via a change in the light sources associated with the access door releases **314**.

Additionally, according to some embodiments, the threshold intensity used in this system test is based on the expected level of luminescence from the spores **181** after the sterilization cycle. For example, given the number and type of spores **181** on the spore carrier **185**, the threshold intensity level for this test may be based on a percentage of the expected level of luminescence assuming all spores **181** were killed during the sterilization cycle (and thus released their DPA prior to germinant release). As the dead spores **181** would be expected to luminesce and return an intensity signal relatively quickly upon contact with the germinant **165**, a lower than expected luminescence intensity may indicate a failure of the germinant **165** to fully release and properly saturate the spore carrier **185**. The threshold intensity (or threshold percentage of the expected luminescence intensity) is not particularly limited so long as it is sufficiently high to accurately determine whether the germinant **165** was properly released. In some embodiments, the threshold intensity may be set to 2000, i.e., out of the range of 0-65535 levels (for a 16-bit image). However, it is understood that in some embodiments, the BI reader **300** does not detect or capture images of a background (or expected luminescence). In such embodiments, the threshold intensity in this test would be set to 0, or this test would be omitted.

Assuming the germinant release system test described above passes, the control system directs the BI reader **300** to continue with the detection cycle. During the detection cycle, the optical assembly may emit light from the excitation source into the BI **100** in each occupied bay, and capture multiple images of the luminescence emitted back through the imaging window **190** and the BI window **379**, as discussed above, and In some embodiments, to determine whether there are live spores, the control system may generate a signal-to-noise ratio, comparing any received luminescence signal to the static background image (when present). In particular, if any spores **181** remained viable after the sterilization procedure, the luminescence emitted back initially may be below an anticipated threshold. The live spores **181**, then, would release their DPA after germination (i.e., sometime after initial contact with the germinant solution **165**), at which time, the newly released DPA would bind with the photoluminescent component of the germinant solution and luminescence (upon activation with the light from the excitation source). However, as this luminescence signal occurs after the live spores have had the time to germinate, this live spore signal does not appear until after the static background image (when present) has been established. As such, any signal from a live spore will appear above the static background signal (when present) or as a

time-lapsed signal, and be identified by the control system as indicative of a live spore, and therefore sterilization failure.

To ensure that the indication of sterilization success or failure is accurate, the entire spore carrier is assessed over time to determine whether any live spores remain. More specifically, while the scan head assembly **350** is positioned under an occupied BI bay **375**, the excitation source emits light on the spore carrier, and the camera captures multiple images of the entire spore carrier. These images captured by the camera assembly **360** are then transmitted to the control processor which may analyze each of the images, e.g., to compare signal to noise (or background) for the returned images. In some embodiments, for example, the processor analyzes each of the captured images pixel-by-pixel. This analysis of the captured images pixel-by-pixel enables quantification of the number of live spores, thus providing a more accurate assessment of sterilization efficacy. In particular, when a spore releases DPA (either from being killed during the sterilization cycle or from germination), the DPA typically releases close to the spore. However, the DPA released by dead spores **181** have had sufficient time to diffuse over the spore carrier **180** by the time the BI **100** is being processed. In contrast, DPA is released by live spores **181** in real time (e.g., in 15 second intervals) and the DPA does not have sufficient time to diffuse away from its pixel location. Thus, the DPA signal from a live spore **181** appears as a local intensity perturbation. As such, the imaging and analysis protocols described herein enable imaging of individual spores on the spore carrier by looking at each pixel on the spore carrier **180**. With a known number of pixels and known number of spores **181** on the spore carrier **180**, the number of live spores **181** can be quantified by the control processor. To that end, the number of pixels is not particularly limited, but in some embodiments, each image may contain 160×160 pixels.

As noted above, when one or more spores remain viable after the sterilization cycle, they will generate a luminescence signal later in time than BI activation, or later in time than the signal generated by dead spores (which contribute to the background signal, when present). Accordingly, in some embodiments, the optical assembly may be configured to capture images at each BI bay **375** at regular time intervals. The length of each interval is not particularly limited, but should be long enough to capture multiple images of the spore carrier during each stop at the respective BI bay **375**. For example, in some embodiments, each interval may be about 3 seconds long, such that when the scan head assembly **350** stops at an occupied bay **375**, it remains there for 3 seconds, emitting light onto the spore carrier, and capturing an image of the luminescence returned by the spore carrier, such image being an accumulation of photons captured over thousands of exposures. More specifically, in some embodiments, the linear guide block **343** (driven by the stepper motor and belt drive) rides on the guide rail **343***a* until it reaches the first occupied bay **375**. When it reaches the first occupied bay **375**, the linear guide block **343** is stopped there for the time interval (e.g., for 3 seconds). After this time interval passes, the linear guide block **343** is moved again along the guide rail **343***a* until it reaches the next occupied bay **375**, where it is stopped again for the time interval. This continues until all occupied bays **375** are visited by the scan head assembly. And when the scan head assembly **350** reaches the last occupied BI bay **375**, it returns to the first occupied bay **375** for a second time interval (which is usually equal in length to the first time interval, but may vary if desired), and then cycles through the remaining occupied bays again. The scan block assembly **350** may be operated in this cycling mode for any number of cycles such that each occupied bay **375** undergoes multiple illumination and image capture cycles during each detection cycle of the BI reader **300**. This time-gated imaging of the spore carrier enables the BI reader **300** and the control processor to compare the time-gated images to each other, and detect any luminescence signals appearing at different times, or appearing above the initially established background image (when present). As discussed above, when coupled with the pixel-by-pixel analysis of these images, this allows the BI reader **300** to detect individual spores on the spore carrier, and to quantify the number of spores that remained alive after the sterilization procedure. It is understood that the occupied bays **375** of the reader **300** may be analyzed in this manner in any order, including, e.g., beginning the scan head assembly cycles from a left-most bay, a right-most bay, or a bay somewhere in the middle.

According to embodiments, the BI reader **300** can complete a full detection cycle (i.e., including multiple cycles of the scan head assembly **350**) in about 15 minutes or less. As discussed above, the positioning assembly **340** may move the scan head assembly **350** beneath various of the BI bays **375** for relatively brief intervals, and may cycle through each of the BI bays **375** multiple times during one detection cycle. As such, multiple images at each BI bay **375** are captured, which provides a history of images over time. The BI reader **300** may be configured to analyze patterns at each biological indicator **100** over time, reducing the likelihood of noise providing a false negative, thereby improving reliability of the BI reader **300**. According to embodiments, when a live spore **181** is detected in one of the BI bays **375**, the detection cycle may be stopped, or the BI bay **375** may be omitted during continued testing of other BI bays **375** for any live spores **181**.

After the detection cycle of the BI reader **300** is complete, the BI reader **300** may output a reading or indication to the user, indicating whether each of the tested biological indicators **100** had any live spores. The reading or indication output by the reader may be either via information displayed on the display **312** and/or via a change in the light sources associated with the door releases **314**. For example, if the reading or indication is that a BI **100** did test positive for live spores during the detection cycle (and therefore that the sterilization cycle associated with that BI failed), the BI reader **300** may identify the bay number on the display next to an indication such as "fail," or any other indication that tells the user that the sterilization cycle associated with that BI was not successful. Additionally or alternatively, the light source corresponding to the BI bay may change, e.g., from off to on (or vice versa), from one color to another (e.g., from green to red, or vice versa), from on to flashing, etc. Also additionally or alternatively, the BI reader **300** may include an audio alarm that may sound in the event of detection of a live spore (or in the case of a system fault, as discussed above). Similarly, if no live spores were detected during the detection cycle (thereby indicating that the sterilization cycle was successful), the reader **300** may identify the bay number on the display next to an indication such as "pass," or any other indication that tells the user that the sterilization cycle associated with that BI was successful. Additionally or alternatively, the light source corresponding to the BI bay may change, e.g., from off to on (or vice versa), from one color to another (e.g., from red to green, or vice versa), from on to flashing, etc. Also additionally or alternatively, the audio alarm may sound, e.g., with a distinct sound indicating success (whereas a different sound may be used to indicate

failure of the sterilization cycle, and another different sound may be used to indicate a system fault).

When the detection cycle is complete, the solenoid **405** is retracted, releasing the shuttle **420**, which is retracted toward the rear panel **391**, thus moving the door interlock spring **421** away from the retaining clip **319**, and unlocking the access door **313** at the hook portion **313***c*. As the shuttle **420** is retracted, the germinant release lever **401** is released and the push rod **403** is retracted from the opening **121** in the biological indicator **100**. The user may then depress (or otherwise actuate) the access door release **314**, which releases the access door **313**, allowing for removal of the biological indicator **100**. The secondary spore carrier may then be removed from the biological indicator **100** and used to run a reference culture test to verify the results returned by the BI reader **300** (if necessary).

While certain exemplary embodiments of the present disclosure have been illustrated and described, those of ordinary skill in the art will recognize that various changes and modifications can be made to the described embodiments without departing from the spirit and scope of the present invention, and equivalents thereof, as defined in the claims that follow this description. For example, although certain components may have been described in the singular, i.e., "a" germinant compound, and the like, one or more of these components in any combination can be used according to the present disclosure.

Also, although certain embodiments have been described as "comprising" or "including" the specified components, embodiments "consisting essentially of" or "consisting of" the listed components are also within the scope of this disclosure. For example, while embodiments of the present disclosure are described as comprising a BI housing, a germinant container, a germinant releaser, a germinant releaser support, a first spore carrier, and an imaging window, embodiments consisting essentially of or consisting of these components are also within the scope of this disclosure. Accordingly, a biological indicator may consist essentially of a BI housing, a germinant container, a germinant releaser, a germinant releaser support, a first spore carrier, and an imaging window. In this context, "consisting essentially of" means that any additional components or process actions will not materially affect the product or the results of the detection cycle (e.g., of the system or BI reader).

As used herein, unless otherwise expressly specified, all numbers such as those expressing values, ranges, amounts or percentages may be read as if prefaced by the word "about," even if the term does not expressly appear. Further, the word "about" is used as a term of approximation, and not as a term of degree, and reflects the penumbra of variation associated with measurement, significant figures, and interchangeability, all as understood by a person having ordinary skill in the art to which this disclosure pertains. Any numerical range recited herein is intended to include all sub-ranges subsumed therein. Plural encompasses singular and vice versa. For example, while the present disclosure may describe "a" germinant compound, a mixture of such compounds can also be used. When ranges are given, any endpoints of those ranges and/or numbers within those ranges can be combined within the scope of the present disclosure. The terms "including" and like terms mean "including but not limited to," unless specified to the contrary.

Any numerical value inherently contains certain errors necessarily resulting from the standard variation found in their respective testing measurements. The word "comprising" and variations thereof as used in this description and in the claims do not limit the disclosure to exclude any variants or additions.

What is claimed is:

1. A biological indicator (BI) comprising:

a BI housing;

a germinant container inside the BI housing and housing a germinant composition;

a germinant releaser configured to release the germinant composition from the germinant container;

a germinant releaser support supporting the germinant releaser and configured to bring the germinant releaser into contact with the germinant container upon application of a force to the germinant releaser support or the germinant container, the germinant releaser support comprising:

a seat defining a germinant releaser opening which receives the germinant releaser; and

a plurality of support legs which support the seat and are configured such that the seat is located above the germinant container, the plurality of support legs having flexibility to allow for movement of the seat when downward pressure is applied to the seat;

a first spore carrier inside the BI housing, the first spore carrier having a plurality of spores deposited at a first surface thereof; and

an imaging window at a first surface of the BI housing.

2. The biological indicator of claim **1**,

wherein the BI housing defines an opening at a second surface thereof, the second surface opposite to the first surface, the opening configured to receive a germinant release means of a BI reader during BI activation for releasing the germinant from the germinant container,

wherein the opening is located above the germinant releaser along a thickness direction of the biological indicator, and

wherein the opening, the germinant container, the germinant releaser, the spore carrier, and the imaging window are all stacked along the height direction.

3. The biological indicator of claim **1**, wherein the germinant container comprises a glass ampoule or an outer container having a hollow interior sealed by a barrier.

4. The biological indicator of claim **1**:

wherein the first spore carrier is generally planar and has a first side and a second side, said first surface of the first spore carrier being on the first side such that the first spore carrier carries said plurality of spores on the first side thereof,

the first side of the first spore carrier with the plurality of spores thereon being positioned parallel to the imaging window for viewing through the imaging window, and

the germinant composition being configured to contact the first spore carrier and the first plurality of spores thereon after BI activation.

5. The biological indicator of claim **1**, wherein:

the first spore carrier is positioned between a germinant pad and the imaging window, and

the first spore carrier, the germinant pad, and the imaging window are all positioned in a stacked arrangement as substantially parallel planes.

6. The biological indicator of claim **1**, further comprising a germinant pad positioned adjacent to the first spore carrier,

wherein the germinant releaser is movable towards the first spore carrier and the germinant pad during BI activation to release the germinant composition from the germinant container, and the germinant releaser being configured to press the germinant pad against the

first spore carrier towards the imaging window at least during BI activation to hold the germinant pad and first spore carrier in place.

7. The biological indicator of claim **1**, wherein at least the first surface of the first spore carrier comprises a planar surface holding the plurality of spores which first surface is gray or black in color.

8. A process challenge device for use in determining the efficacy of a sterilization cycle, the process challenge device comprising:

a tray defining a first cavity and a tab;

a closure portion configured to be attached to the tray to seal the first cavity;

a sterilant sterilization integrator; and

a sterilant access port,

the first cavity containing the biological indicator of claim **1** and the sterilant sterilization integrator, the tab being configured to separate the biological indicator and the sterilant sterilization integrator.

9. A biological indicator (BI) comprising:

a BI housing;

a germinant container inside the BI housing and housing a germinant composition;

a germinant releaser configured to release the germinant composition from the germinant container;

a germinant releaser support supporting the germinant releaser and configured to bring the germinant releaser into contact with the germinant container upon application of a force to the germinant releaser support or the germinant container;

a first spore carrier inside the BI housing, the first spore carrier having a plurality of spores deposited at a first surface thereof; and

an imaging window at a first surface of the BI housing,

the BI housing defining an opening located above or adjacent the germinant releaser, the opening being configured to receive a germinant release means of a BI reader during BI activation for releasing the germinant from the germinant container,

the BI housing further comprising a sterilant entry port at a location different from the opening, and

prior to BI activation, the opening is sealed by a sealant material to exclude sterilant, and the sterilant entry port is open to receive sterilant.

10. The biological indicator of claim **9**, further comprising a germinant pad, the first spore carrier being positioned between the germinant pad and the imaging window, and

the first spore carrier, the germinant pad, and the imaging window are all positioned in a stacked arrangement as substantially parallel planes.

11. The biological indicator of claim **10**, wherein the germinant releaser is movable towards the first spore carrier and the germinant pad during BI activation to release the germinant composition from the germinant container, and the germinant releaser being configured to press the germinant pad against the first spore carrier towards the imaging window at least during BI activation to hold the germinant pad and first spore carrier in place.

12. The biological indicator of claim **9**, wherein at least the first surface of the first spore carrier is gray or black in color.

13. A biological indicator (BI) comprising:

a BI housing;

a germinant container inside the BI housing and housing a germinant composition;

a germinant releaser configured to release the germinant composition from the germinant container;

a germinant releaser support supporting the germinant releaser and configured to bring the germinant releaser into contact with the germinant container upon application of a force to the germinant releaser support or the germinant container;

a first spore carrier inside the BI housing, the first spore carrier having a plurality of spores deposited at a first surface thereof; and

an imaging window at a first surface of the BI housing,

the BI housing comprising a grip portion and a protrusion portion, the grip portion and protrusion portion being lateral to each other along a length dimension of the biological indicator, the protrusion portion being configured to house at least a portion of the germinant container, the germinant releaser, the germinant releaser support and the first spore carrier.

14. The biological indicator of claim **13**, further comprising a germinant pad, the first spore carrier being positioned between the germinant pad and the imaging window,

the first spore carrier, the germinant pad, and the imaging window are all positioned in a stacked arrangement as substantially parallel planes, and

in the activated position, the germinant releaser or germinant releaser support presses the first spore carrier and the germinant pad towards the imaging window.

15. The biological indicator of claim **13**,

wherein the imaging window is on a bottom surface of the BI housing, and the first surface of the first spore carrier and the plurality of spores thereon are oriented downward towards the imaging window; and

wherein the BI housing has side walls which are opaque.

16. The biological indicator of claim **15**, further comprising an insertion groove on an exterior surface of at least one side wall of the BI housing for engagement with a BI reader.

17. The biological indicator of claim **13**, further comprising an opening in the protrusion portion of the BI housing opposite the imaging window, and a sealant material sealing the opening prior to BI activation, and which sealant is configured to be broken during BI activation.

18. The biological indicator of claim **13**, wherein the protrusion portion of the BI housing comprises an opening in the BI housing located above the germinant releaser along a thickness direction of the protrusion portion, and the opening, the germinant container, the germinant releaser, the first spore carrier, and the imaging window are all stacked along the height direction in the protrusion portion.

19. A biological indicator (BI) comprising:

a BI housing having a first surface and second surface opposite the first surface, the BI housing defining an opening at the second surface, the opening being located in an area adjacent a first end of the BI housing, and the biological indicator further comprising a sterilant opening at a second end of the BI housing, the second end opposite to the first end;

a germinant container inside the BI housing and housing a germinant composition;

a germinant releaser configured to release the germinant composition from the germinant container;

a germinant releaser support supporting the germinant releaser and configured to bring the germinant releaser into contact with the germinant container upon application of a force to the germinant releaser support or the germinant container;

a first spore carrier inside the BI housing, the first spore carrier having a plurality of spores deposited at a first surface thereof; and

an imaging window at a first surface of the BI housing.

20. The biological indicator of claim **19**, wherein:

the opening is located above the germinant releaser along a thickness direction of the biological indicator, and

the opening, the germinant container, the germinant releaser, the spore carrier, and the imaging window are all stacked along the height direction.

21. The biological indicator of claim **19**, wherein the opening is aligned with the germinant container and the first spore carrier,

the opening being sealed against sterilant entry via the opening prior to BI activation, and the opening being configured to receive a germinant release means of a BI reader during BI activation for releasing the germinant from the germinant container.

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